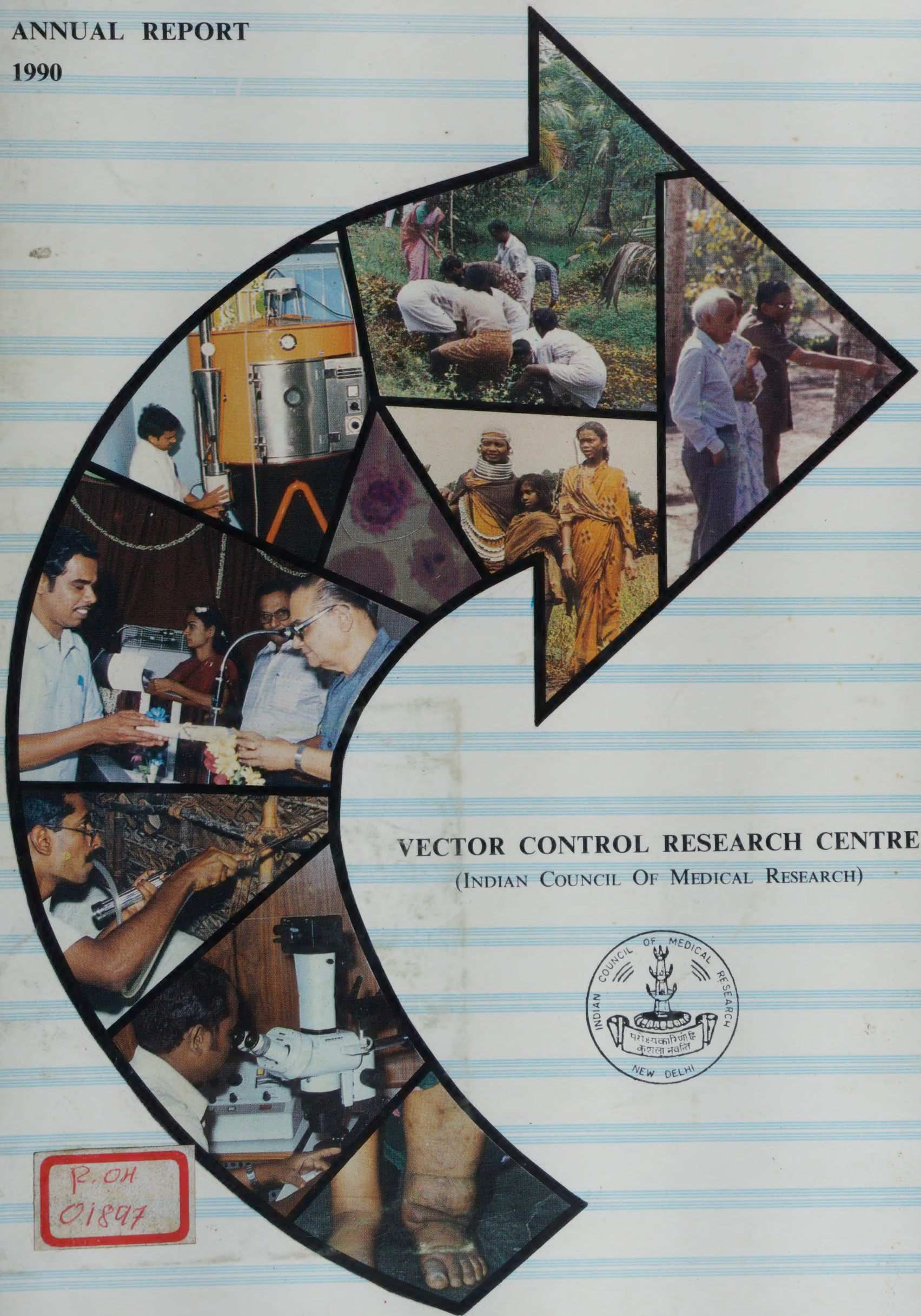
ANNUAL REPORT

1990



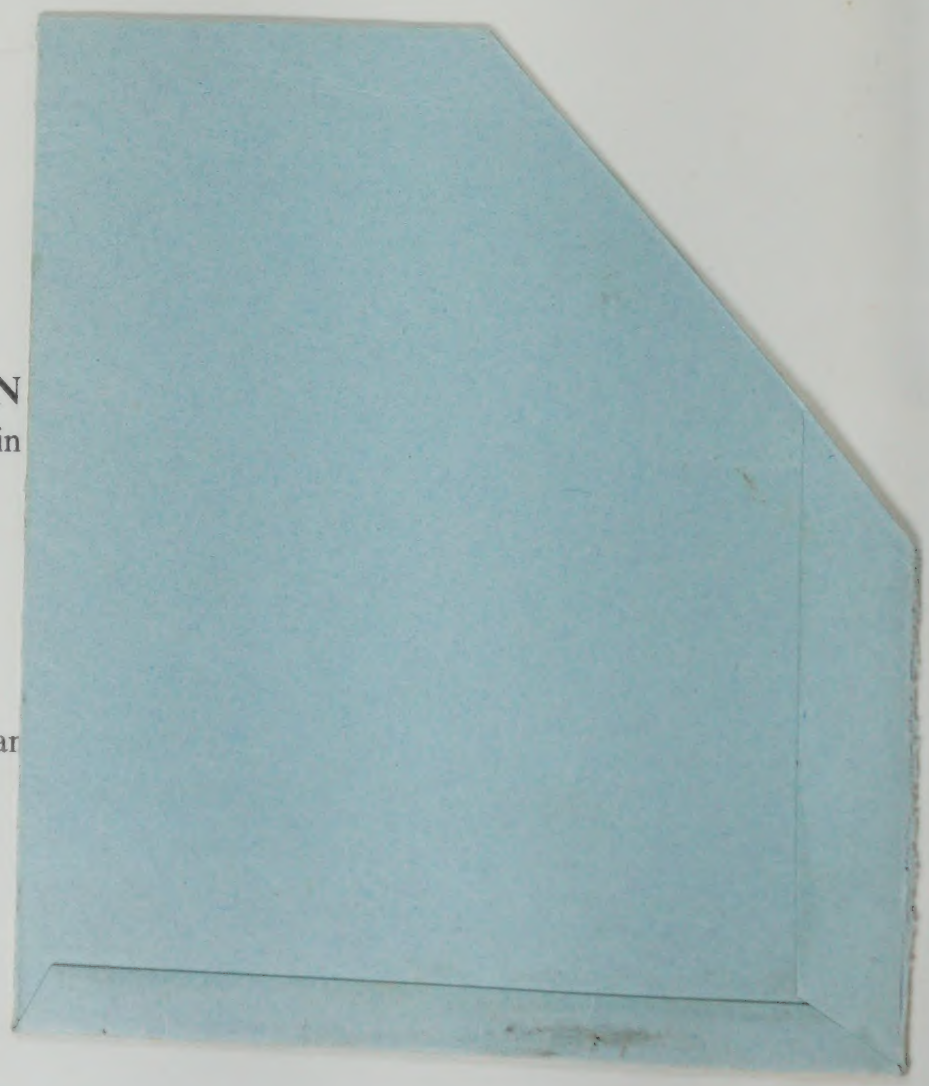
VECTOR CONTROL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

WORLD HEALTH ORGANIZATION
Collaborating Centre for Research & Training in
INTEGRATED METHODS OF VECTOR CONTROL

THE COVER:

Our commitment towards better control and mar
and vector-borne diseases.



VECTOR CONTROL RESEARCH CENTRE

PONDICHERRY 605006
INDIA

ANNUAL REPORT
1990

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CONTENTS

I. PREFACE

In the year 1990 the Vector Control Research Centre completed 15 years of its existence. The year also saw another significant development when Dr.P.K.Rajagopalan retired from the office of Director on 31st October. This is a traumatic experience for the Centre, as Dr.Rajagopalan's name had become almost synonymous with that of the Centre. He had guided its destiny through thick and thin almost from its inception. It was mainly due to his foresight, dynamism and ceaseless struggle over the years that this Centre has come to occupy a place of pride in the scientific community not only within the country, but also internationally. We wish Dr.Rajagopalan a happy and peaceful retired life.

From its inception in 1975, the Centre has identified Filariasis as a major area of field research. Extensive data have since been collected on all aspects of ecology of the vector *Culex quinquefasciatus* and, during the recent years on *Mansonia* species. Studies on clinical and epidemiological aspects are also underway. Two major control projects have been undertaken by the Centre. One was in Pondicherry, which was based mainly on environmental and other integrated methods of vector control. The second is currently in progress in Shertallai, in Kerala State, which incorporates community participation as its main component and has a multifaceted approach. In a disease like filariasis, it will take time to feel the impact of these measures, although declining trends in the areas of operation have already been noticed.

Our Pondicherry experience had shown that there were difficulties in transferring the technology developed in the course of implementation of the project, to local (health) authorities in spite of the availability of adequate resources. Therefore, in Shertallai, the VCRC ventured to pass on the appropriate technology for filariasis control directly to the community. Taking advantage of the high literacy and community acceptance of the programme, an attempt is made to evolve a life style which will not only enhance the economy of the community, but also help in achieving filariasis control.

In accordance with the recommendations of the Scientific Advisory Committee, the Centre is also planning to extend the survey for filariasis, initially to the rest of Alleppey district and then to the entire state of Kerala, particularly to the coastal region, so that both malayan and bancroftian filariasis in the state can be mapped out. The Shertallai project received commendation from Prof.A.S.Paintal, Director General of the I.C.M.R., when he visited the field station and saw the work himself. His visit was a morale booster for the dedicated band of young men and women, who are working on the project.

With the continuing collaboration from Imperial College, London, studies are on way to develop quantitative understanding of parasite dynamics both in vector and human population. Preliminary models have been developed for understanding the relationship of microfilaraemia and clinical disease. Studies on chemotherapy have revealed that a single course of DEC (as advocated by WHO) fails to clear parasitaemia in about 40% of carriers and there is an urgent need to develop rational therapeutic regimen.

Several research groups in different parts of India and abroad are currently studying the problem of filariasis. To bring them together, an International Seminar on Future Research Needs in Lymphatic Filariasis was held at the Centre from 8th to 10th October, 1990. The seminar was inaugurated by Dr. S.P. Tripathy, Additional Director General of the ICMR. In his inaugural address, Dr. Tripathy, stated that the VCRC had now grown from its original role as a Centre on vector control to a much larger role of disease control. He stated that it could now rightly be called as National Institute of Filariasis Research, a name that would be acceptable to the Council and the Government without hesitation. However, the VCRC had preferred the original name by which it is known the world over. Twenty two leading scientists from different parts of India and five from abroad participated. It provided an excellent opportunity to the young and enthusiastic scientists of the Centre to mingle with the mighty and to know the latest in different aspects of filariasis research all over the world. The Seminar was a grand success. It also paved the way for an ensuing WHO/ICMR meeting on field research investigations in lymphatic filariasis in India, which is scheduled for the end of January 1991, and

will focus on the use of Ivermectin, in clinical and field trials in India.

The Koraput project on malaria which was initiated in 1986 has provided some useful but intriguing information on the epidemiology of the disease. One peculiar feature is the fact that even though parasite incidence in some areas around Jeypore is high, gametocyte index is relatively low. Correspondingly, the sporozoite rate in mosquitoes was also low. Unlike Jeypore area, the gametocyte index in man and sporozoite rate in the mosquitoes in Malkangiri was higher. The reason for the low rate of sporozoite in the natural mosquito population in Jeypore area, relative to the incidence of parasite in human population is still not clearly understood. The basic question that needs answer is, "Are we dealing with a problem of malaria persistence with low level transmission or are our techniques inadequate to detect ongoing high level transmission ?"

In an area where many species of mosquitoes have been incriminated as vectors of malaria on the basis of field data, it is important to determine the relative role of each species involved. For this purpose, information on the relative susceptibility of these species to infection with the parasite will be crucial. Therefore, laboratory studies to determine the relative susceptibility of the incriminated species of mosquitoes to different species of Plasmodia occurring in the area are also being planned.

The continued search for new strains of biocontrol agents has yielded new serotypes of *Bacillus thuringiensis* and *B. sphaericus*, new strains of *Lagenidium.* sp. and a streptomycete. A new strategy for producing a higher yield of cell mass of *B. thuringiensis* employing Fed-batch process was developed. An avidin-biotin based ELISA for quantifying the toxin of *B. sphaericus* was developed. This assay could detect *B. sphaericus* toxin to μg level and hence will be helpful in monitoring the toxin level during fermentation and in residual analysis in field. Investigations on the by-products of biopesticides viz. Cyclosprin A (from *Tolypocladium* sp.) and L-dopa (from *B. thuringiensis* H.14) have helped in understanding their biosynthesis, improving yield and checking the activity.

A large scale field trial using a VCRC formulation of *B. sphaericus* is currently being planned. Besides, a proposal for a WHO funded project has been submitted for the clearance of the Govt. of India for a field trial using *B. sphaericus* formulations for the control of mosquitoes in Cochin.

Studies on Quantitative Structure Activity Relationships (QSAR) in pyrethroid esters in relation to physicochemical substituent parameters like lipophilicity, electronic and steric factors made it possible to design compounds with enhanced biological activity. Studies on controlled release formulations led to the development of two formulations (an insect repellent and a mosquito larvicide) which could significantly increase the active life of the active ingredient thereby reduce the cost of the operational programmes. To monitor resistance in mosquito vectors, discriminating dosages of five larvicides and five adulticides were determined. Three new insecticides have been evaluated for their biological activities under the World Health Organization Pesticide Evaluation Scheme (WHOPES).

The fourteenth meeting of the Scientific Advisory Committee was held on 22nd February 1990 and was attended by Dr.P.K.Ramachandran, Emeritus Scientist, DRDE, Gwalior (Chairman), Dr.M.K.K.Pillai, Professor of Zoology, Delhi University, Delhi, Dr.N.Ramakrishnan, National Fellow, Indian Agricultural Research Institute, New Delhi, Dr.V.Sambasivam, Retd. Director of Health Services, Govt. of Pondicherry, Pondicherry, Dr.N.L.Kalra, Central Coordinating Officer, National Malaria Eradication Programme, Delhi, Dr.D.A.P.Bundy, Director, Parasite Epidemiology Research Group, Imperial College, London (Special Invitee) and Dr.B.T.Grenfell, Parasite Population Ecology Group, University of Cambridge, U.K. (Special Invitee). Efforts are being made to implement the recommendations of the committee.

In conclusion, I would once again like to pay a tribute to the outgoing Director, Dr. P.K. Rajagopalan, for building up an excellent team of outstanding scientists whose multifarious contributions in the field of vector control research have been inferior to none. Their enthusiasm shows that the future of the Centre is as bright as its past.

Dr. Vijai Dhanda
Director

II. STAFF POSITION AS ON 31st DECEMBER 1990

DIRECTOR	:	Dr. Vijai Dhanda, M.Sc., Ph.D., D.A.P.&.E (Lond.) #
DEPUTY DIRECTOR	:	Dr. P.K. Das, M.Sc., Ph.D.
ASSISTANT DIRECTOR	:	Dr. K. Balaraman, M.Sc., Ph.D. Dr. K.N. Panicker, M.Sc., Ph.D. Dr. S.G. Suguna, M.Sc., Ph.D. Dr. S.P. Pani, M.D., Ph.D.
SENIOR RESEARCH OFFICER	:	Dr. M. Kalyanasundaram, M.Sc., Ph.D. Dr. S. Sabesan, M.Sc., Ph.D. Dr. P. Jambulingam, M.Sc., Ph.D. Dr. N. Arunachalam, M.Sc., Ph.D.
RESEARCH OFFICER/ SENIOR TECHNICAL OFFICER	:	Dr. T. Mariappan, M.Sc., Ph.D. Dr. K. Krishnamoorthy, M.Sc., Ph.D Dr. S.L. Hoti, M.Sc, Ph.D. Mr. K. Gunasekaran, M.Sc. Dr. Lalit Kumar Das, M.B.B.S. Dr. (Ms) P. Govardhini, M.B.B.S*. Dr. (Mrs.) Prathiba Jayasimhan, M.B.B.S.* Dr. S.S.S. Mohapatra, M.B.B.S.** Mr. M. Kuppusamy, M.Sc. Dr. S. Bhaskaran, M.Sc., Ph.D. Dr. S. Radhakrishnan, M.B.B.S.*
ASSISTANT RESEARCH OFFICER/ TECHNICAL OFFICER	:	Mrs. A. Manonmani, M.Sc. Dr. K.D. Ramaiah, M.Sc., Ph.D. Mr. Dominic Amalraj, M.Sc. Mr. N. Pradeep Kumar, M.Sc. Mr. G. Rajendran, M.Sc. Mr. K. Viswam Mr. N. Somachary Dr. K.P. Paily, M.Sc., Ph.D. Mr. A.R. Rajavel, M.Sc.,M.Phil. Mr. R. Srinivasan, M.Sc. Mr. S. Subramanian, M.Sc. Mr. N. Balakrishnan, M.Sc. Mr. P. Vanamail, M.Sc. Mr. Sarat Kumar Parida, M.Sc.

Dr. P.K. Rajagopalan, M.Sc., Ph.D., M.P.H.(Calif.), FIBiol.(Lond), relinquished the office of the Director of the Centre on 31st Oct. 1990 after superannuation. Dr. Vijai Dhanda, took over charge of the post of Director on 1st Nov. 1990.

RESEARCH ASSISTANT	:	Ms. V. Vasuki, M.Sc., M.Phil. Mrs. Nisha George, M.Sc. Mr. C. Sadanandane, M.Sc. Mr. Sudhansu Sekar Sahu, M.Sc. Mrs. B. Nanda, M.S.W. Mrs. Ambili Kumar, M.A. Ms. M. Jayasree, M.Sc. Mr. M.P. Prasad, M.Sc. Mr. V. Vijayan, M.Sc. Mr. Kailash Prasad Patra, M.Sc. Ms. R. Shanthi, M.S.W. Ms. Abidha, M.Sc. Mrs. K.S. Snehalatha, M.A. Ms. A. Krishnakumari, M.A. Mr. K. Govindan, B.Sc.* Dr. Vijayakumar, M.A., Ph.D.
STATISTICAL ASSISTANT	:	Ms. A. Srividya, M.Sc. Mr. A. Manoharan, M.Sc.
JUNIOR RESEARCH FELLOW	:	Mr. K. Gopala Rathinam, M.Sc., M.Phil. Mr. K. Anand Kumar, M.Sc. Mr. C. Sekar, M.Sc., M.Phil.

ADMINISTRATION & ACCOUNTS

ADMINISTRATIVE OFFICER	:	Mr. N. Premkumar, B.Com.
ACCOUNTS OFFICER	:	Mr. S. Swaminathan
SECTION OFFICER	:	Mr. S. Chandrasekaran, B.Com.
SUPERINTENDENT	:	Mr. V. Vijayamoorthy, B.Com.
SENIOR LIBRARIAN	:	Mrs. R. Sundarammal, B.Sc.,B.Lib.

* Project Staff
** Seconded by R.M.R.C., Bhubaneswar.

III. RESEARCH STUDIES

1. STUDIES ON MALAYAN FILARIASIS AND ITS CONTROL IN SHERTALLAI, KERALA STATE

The Director-General of Indian Council of Medical Research, Prof. A.S. Paintal and the Additional Director General, Dr. S.P. Tripathy visited Shertallai field station during the early part of the reporting year (Plate I, A&B). Both of them have recorded their full satisfaction on the line and pace in which the project is progressing. During a civic reception in his honour, the DG has assured the people of Shertallai that VCRC will remain in Shertallai till the objective of elimination of malayan filariasis from Kerala is totally achieved. The ADG has suggested the expansion of the programme to cover the entire *Brugia malayi* belt in a phased manner. He has also emphasised the importance of community action in the control of filariasis and highly appreciated the activities of FILCO movement. The advice and suggestions of the DG as well as ADG are being fully incorporated in the implementation of the programme.

1.1. ECOLOGY AND POPULATION DYNAMICS OF *MANSONIOIDES*:

In-depth ecological studies of *Mansonioides* mosquitoes formed a major thrust area of activities of Shertallai field station.

1.1.1. Ecology of immatures:

Various aspects of larval ecology such as biotic composition, spatial distribution of *Mansonioides* larvae, interspecific association of immatures, estimation of the Productivity Index and Breeding Potential of different breeding habitats, comparison of different sampling procedures, host plant preferences for oviposition and larval attachment, etc. were extensively investigated.

Both quantitative and qualitative assessment of the planktonic organisms in pond habitat in relation to *Mansonioides* breeding were made. The number of planktonic organisms in ponds (n = 124) supporting *Mansonioides* breeding (mean = 171 $\pm$ 104) was found to be significantly higher (T = 3.87; P = 0.002) when compared to ponds without breeding (mean = 117 $\pm$ 66). In the vector breeding ponds, the planktons belonging to Oxyphotobacteria, Sarcomastigophora, Ciliophora, Bacillariophyta, Gastrotricha and Rotifera were present in large numbers.

The number of *Mansonioides* larvae recorded from the samples collected near the pond margin ranged from 1 to 233, with a mean of 45. 74 per dip, while it was 1 to 299 with a mean of 47.5 from the centre of the pond. Statistical analysis (one way variance test) showed that the per dip density did not differ significantly ($P > 0.05$) between the density recorded near the margin and centre of the pond, indicating a uniform distribution of larvae in the pond habitat.

A high degree of association between *Mansonia annulifera* and *M. uniformis* was noticed in ponds (C_{AB} value = 0.3273 $\pm$ 0.044) as well as fallow lands (C_{AB} value = 0.8760 $\pm$ 0.083). However, the association was relatively higher in fallow lands when compared to the ponds. A similar degree of association between these two species was also found from the values of Index of Association (I) in both the habitats [(I) value for ponds = 0.055; fallow lands = 0.873]. In fallow lands the value was found to be more than 15 times higher when compared to pond habitat. This is because of prevalence of a wide range of host plants in fallow lands than in pond habitat, and a relatively higher incidence and intensity of *M. uniformis* breeding in fallow lands, as they are less restricted to their choice of host plants.

Productivity Index and Breeding Potential of different breeding habitats were assessed in relation to their nature and type of hydrophytes' prevalence. Among the different breeding habitats, a relatively higher Productivity Index (PI) was recorded for the fallow lands (1.288) and ponds (0.386), when compared to channels (0.0266), paddy fields (0.0289), canals (0.0004) and lake (0.0004). The mean number of pupae recorded from fallow lands and ponds were 0.718 and 0.272 per dip respectively. The percentage of dips positive for pupae was found to be the maximum (17.95%) in fallow lands followed by ponds (14.19%).

2.

The Breeding Potential (BP) was estimated to be 1829.81 for fallow lands, 1431.69 for ponds, 31.12 for channels, 15.03 for paddy fields, 0.16 for lake and 0.07 for canals. The prevalence of hydrophytes in fallow lands for a restricted period of seven months makes it seasonal, whereas ponds remain perennial and therefore the major breeding habitat in the contribution of *Mansonioides* in the study area. Though seasonal, fallow lands ranked as the second most important habitat.

Analysis on the Productivity Index and Breeding Potential of pond habitat in relation to the type of hydrophytes showed that polluted pond habitat infested with mixed vegetation recorded the maximum values of PI (45.1) and BP (3242.19). Clean ponds with mixed vegetation ranked second in their contribution of *Mansonioides* (PI = 16.41; BP = 1332.86). Though the Productivity Index of polluted ponds with *P. stratiotes* was nearly three fold of that recorded in clean ponds with *P. stratiotes*, the Breeding Potential was three times higher in the latter. Thus, among the pond habitat, polluted ponds infested with mixed vegetation was estimated to contribute to the maximum production of *Mansonioides*.

Measurements of width of larval head capsule were used as an indicator of growth of immature stages of *M. annulifera*. The mean width of the head capsule in I, II, III and IV instar larva was 243.5, 359.0, 659.0 and 979.5 μm respectively and this can be used for instar identification. The growth ratio of head capsule for different instars from its width ranged between 1.474 and 1.836, with a mean of 1.599. This shows that the immatures were observed to grow in allometric manner. Nonsignificant value of pooled chi-square confirms harmonic growth agreeing with Dyar's Law.

A total of 32 aquatic plants was used to study their hospitality for *Mansonioides* larvae. 25 fourth instar larvae were used in each trial with three replicates for each plant. Observations were continued till the emergence of adults was completed. The percentage of larvae successfully completed their development ranged from 0 to 69.33% for different plants used. Out of 32 plants used, 26 were found to support *Mansonioides* larvae while in the rest there was no emergence. Over 50% of emergence was noticed in *Marsilea quadrifoliata, Pistia stratiotes, Isachnaeae miliaceae, Monochoria vaginalis, Certatoipteris thalictroides* and *Jussaiea repens*. The proportion of larvae survived till their emergence was compared between *P. stratiotes,* the known preferred host plant of *M. annulifera* with the rest of the plants that supported larvae. The maximum proportion of emergence of 69.33% was noticed in *M. vaginalis* and it was significantly higher (χ^2 = 4.69; P = 0.03). When compared with *P. stratiotes* no significant difference in the proportion of adults emerged, was noticed in both *M. quadrifoliata* (χ^2 = 0.43; P = 0.057) and *J. repens* (χ^2 = 0.26; P = 0.87), though a higher value of emergence was recorded in the latter two species. Equal proportion of emergence was recorded from *I. miliaceae* and *P. stratiotes*. The proportion of emergence from rest of the plants was significantly ($P < 0.05$) lower when compared with *P. stratiotes*.

The present experiments indicate that among the aquatic plant species prevalent in Shertallai area *M. vaginqlis* appears to be the chief host plant for *M. annulifera* and the other important plants in the order of their degree of hospitality (in terms of percentage emergence) include *M. quadrifoliata, J. repens, C. thalictroides, P. stratiotes* and *I. milliaceae.*

"Uprooting", "Cylinders" and "Cloth dippers" are the three methods generally used for sampling the immatures of *Mansonioides* larvae. An attempt has been made to find out the reliability of "Cloth dipper" method which is followed in Shertallai for routine immature sampling. Trials were made in 23 *P. stratiotes* positive ponds. There was no marked difference in the number of larvae collected following different methods. One way analysis of variance test on per plant density showed that "F" calculated value (0.01) was less than tabulated value (3.23) at 5% level suggesting that there was no significant difference in per plant density of larvae due to different sampling methods. The species composition of the larvae collected by different sampling methods also did not show any difference.

Host plant preference for oviposition by the adult mosquitoes was studied by examining the number of egg clusters in different hydrophytes in ponds, infested with mixed vegetation where the adults have the choice for selecting their host plant for oviposition. The average number of egg clusters per plant was found to be the maximum in *P. stratiotes* plants in both clean and polluted habitats. In *S. molesta* plants it was found to be 22 to 30 times less than that of *P. stratiotes*. Only one egg cluster was obtained out of 1,639 *E. crassipes* examined from ponds infested with mixed vegetation.

Similarly, host plant preference by immatures for attachment was studied in ponds with mixed vegetation. In polluted ponds density of larvae was found to be more on *P. stratiotes* followed by *E. crassipes* and *S. molesta*. Test of significance showed that the density was significantly different between *P. stratiotes* and *S. molesta* but it was not so between *P. stratiotes* and *E. crassipes*.

Slow release formulation of Fenthion:

Fenthion briquettes, a slow release formulation was evaluated against immatures of *Mansonioides* mosquitoes at a dose of 2.25 gram per sq.m. in polluted pond habitat with high larval density. The pretreatment larval density in the treated ponds was 244.5 per dip and in the control ponds it was 271.17 per dip. In all the treated ponds no larva was recorded upto fourth month following treatment. Further observations are in progress.

1.1.2. Ecology of adults:

Vector density:

The average man biting rate (MBR) and the per man hour indoor resting densities (PMD) recorded for *M. annulifera, M. uniformis* and *M. indiana* were 24.67, 12.73 & 0.47 and 3.29. 0.25 & 0.01 respectively. The seasonal biting and resting densities had a linear correlation for both *M. annulifera* ($r = 0.7595$; $P = 0.0042$) and *M. uniformis* ($r = 0.8736$; $P = 0.0002$), the biting density being 7.50 times more than indoor resting density for the former and 9.78 times for the latter species.

M. annulifera was observed to constitute 85.90% of the indoor resting population of *Mansonioides*. However in the outdoor resting population, as estimated by "drop-net cage" (Plate II, A) *M. uniformis* predominated (98.00%). The statistical analysis of the proportion of these mosquitoes in different biotopes showed a significant difference (*M. annulifera*, $Z = 29.75$; $P < 0.05$ and *M. uniformis*, $Z = 31.17$; $P < 0.05$) indicating the endophily of the former and the exophily of the latter species. Analysis of the abdominal condition of the indoor resting and the exit trap collections (Plate II, B) also confirmed the exophily of *M. uniformis*.

House frequenting behaviour of Mansonioides:

The Indoor (human dwellings) resting densities of *Mansonioides* were higher during night hours compared to day. The per man hour resting density (PMD) estimated during night and day hours respectively were 2.54 and 1.94 for *M. annulifera*, 1.12 and 0.44 for *M. uniformis*, and 0.035 and 0.007 for *M. indiana*. There was a significant increase in the density of *M. uniformis* during 20.00 to 21.00 hr ($T = 2.18$; $P = 0.04$) compared to the day time collections.

The analysis of abdominal condition revealed a higher proportion of unfed during night hours for both *M. annulifera* and *M. uniformis*, suggesting their influx during dusk hours. This influx was significantly higher for *M. uniformis* ($Z = 2.99$; $P < 0.05$) unlike that of *M. annulifera* ($Z = 0.94$; $P > 0.05$), thereby it could be inferred that unfed *M. uniformis* enters houses for feeding, from outdoor temporary shelters following oviposition. The higher proportion of fullfed (*M. annulifera* - 0.59 and *M. uniformis* - 0.81) during day hours indicates that a major chunk of fed mosquitoes rest in the nearest available site (indoor) just after feeding. The proportions of fullfed specimens of *M. annulifera* and *M. uniformis* encountered during dusk hours was only 0.21 and 0.15 respectively. This reduction may be attributed to the progression of fullfed to semigravid by that time. However, this difference for *M. uniformis* exceeded the difference of *M. annulifera* suggesting a certain degree of exodus of this species during this period. The proportion of semigravid specimens of both the species increased from 0.37 and 0.17 in day hours to 0.64 and 0.75 during night (17.00 to 19.00) hours. This increase in semigravid proportion was maintained for *M. annulifera* in subsequent hours. However, the proportion of semigravid reduced drastically in the case of *M. uniformis* (Z cal. for 19.00 to 20.00 hours $= 3.18$; $P < 0.05$ and Z cal. for 20.00 to 21.00 hours $= 5.45$; $P < 0.05$) indicating the exodus of this species during the dusk.

From the above observations it could be concluded that *M. uniformis* leaves the houses during the dusk hours on the subsequent night of feeding in search of resting sites outdoor. Inspite of this exodus, the indoor resting density of this species increased during dusk hours owing to the influx of unfed ones which enter houses for feeding.

Further, analysis of abdominal condition of *Mansonioides* obtained from exit trap collections showed that among *M. annulifera*, 70.2% constituted semi-

gravid and gravid mosquitoes which leave the house for oviposition. The proportion of gravids of this species was significantly higher (24.04%) than *M. uniformis* (χ^2 = 4.13; P = 0.04) suggesting a greater endophily of *M. annulifera*. The unfed and fullfed specimens of *M. uniformis* constituted 61.11% of the mosquitoes trapped in exit traps which leave the house for resting outdoor. Interestingly abdominal condition '3' (Sella stage 3) was higher (35.19%) than '2' (16.67%) suggesting that these species rest one day after feeding and leave the house on the subsequent night.

Oviposition periodicity:

The oviposition activity of *M. annulifera* commenced just after sunset and continued till noon on the subsequent day. However, 72.5% of oviposition took place between 21.00 hr and 06.00 hr. The peak of activity (MW = 26.91%) was recorded in the last quarter of the night, i e., 03.00 hr to 06.00 hr.

Population dynamics:

The duration of immatures ranged from 19 (summer months) to 21 (winter months) days. The probability of immature survival was 7.09% for the former, and 8.97% for the latter season. The daily adult survival estimated from parity status varied from 55.03% (May) to 82.45% (January), the average being 78.01%.

The net reproductive rates (R_0) computed from life and fecundity tables constructed using immature duration and survival, fecundity, sex ratio, duration of gonotrophic cycles and adult survival and longevity through different months for *M. annulifera* were more than 'one', indicating an increase in the population in all months except for May (the last month of summer season), and June (the first month of the monsoon season). The mean generation time ('T') ranged from 29.55 (May) to 34.32 days (December). The intrinsic rate of natural increase was maximum (0.0477) in the month of April. The finite rate of increase ranged from 0.99 in May to 1.0490 in April. The doubling time estimated in days varied from 14.52 (April) to 49.50 (September).

Vectorial potential:

Susceptibility status of Mansonioides to Brugia malayi:

Studies were initiated to determine the duration of extrinsic cycle of *B. malayi* and the susceptibility status of *Mansonioides* to this parasite. Out of 100 specimens of *M. annulifera* fed on microfilaria carrier (microfilaria intensity of 28 per mm^3), 41 were found survived and dissection of 5 specimens daily from the day of infection showed an extrinsic incubation period of eight days. All the 41 specimens dissected, were observed to be infected (with mf, first stage, second stage or infective stage in accordance with the day of dissection) revealing a chance of cent percent fed specimens becoming infected and infective. The mean number of microfilaria per mosquito and the mean number of infective larvae per mosquito remained almost same, which were 4.2 and 4.0 respectively. Further studies on these lines are in progress.

Natural infection status and transmission indices:

The natural infection and infectivity rates (%) recorded for *M. annulifera, M. uniformis* and *M. indiana* were 1.61 & 0.41, 1.64 & 0.24 and 1.00 & 0.33 respectively. The indices of transmission such as Annual Infective Biting Rate (AIBR), Annual Transmission Index (ATI), Annual Transmission Potential (ATP) and Risk of Infection Index (RII) computed for the three *Mansonioides* species are given in Table 1.1.

Table 1.1

Species	AIBR	ATI	ATP	RII
M. annulifera	22.85	154.24	157.59	0.00522
M. uniformis	5.50	21.91	16.61	0.00125
M. indiana	1.90	3.80	3.64	0.00043

1.2. FISH, AS BIO-CONTROL AGENT AGAINST *MANSONIOIDES:*

Studies on the biology of Osphronemus goramy (Giant gourami),an important bio-control agent against Mansonioides:

The utility of *Osphronemus goramy* (Giant gourami) in the control of malayan filariasis has already been documented in the annual report of 1989. The reproductive biology of this fish was studied in detail, in view of mass culturing them as they remain an en-

dangered species of fish.

With the advent of the spawning season, the breeding pairs showed an aggressive territorial behaviour. Subsequently the site for nest building was chosen mostly in an area with marginal vegetation or roots of plants protruding from the banks. For the construction of a nest, a pair took 9-24 days, utilising the naturally available marginal vegetation, whereas it took only 2 to 7 days when coconut fibres were offered.

Fecundity, hatchability and fry survival of *O. goramy* are given in table 1.2.

Table 1.2

Nest No.	No.eggs laid	No. hatched	Hatchability	Fry survival	
				Number	%
1.	208	15	7.21	15	100.00
2.	1022	136	13.30	63	45.32
3.	3539	3535	99.88	3390	95.89
4.	1792	1760	98.21	241	13.39
5.	920	22	2.39	11	50.00
6.	577	150	26.00	52	34.66
7.	678	263	38.79	77	29.27
8.	1124	27	2.40	8	29.62
9.	2107	2065	98.00	255	12.34
10.	2054	2022	98.44	263	13.00
Mean	1402	999.50	71.30	437.50	43.70

The eggs were deposited at different layers with more numbers at the bottom (1500 to 2200 nos.)and less towards the surface (200 to 800 nos.). A maximum of four such layers were observed within a single nest. The eggs were semi-transparent and round in shape with an average of 2.5 mm in diameter. Females spawning for the first time produced significantly fewer eggs than the older females. The number of eggs laid in the nests ranged from 208 to 3,539 averaging 1,402. While the average hatchability was 71.35%, the fry survival rate was 43.7%. By fourth month, they reached fingerling stage, when they measured 7 to 9 mm in length and started devouring the leaves of tender *Pistia* plants. Only 13% to 20% of the fingerlings survived to adults. Sexual maturity was attained in a period of 20 to 24 months, when the length and weight varied from 25 cm to 30 cm and 1.5 kg to 2.0 kg respectively.

Parasites of fish and their control:

Members of Branchiuria and Copepoda were found to infest both the adults as well as fingerling stages of *O. goramy* in both nursery and culture ponds. Infestation with *Argulus* was the major problem encountered while culturing this fish. These Copepods were seen attached to the gills and pectoral fins and also on other body parts of fingerlings. Dip treatment in 0.1% acetic acid followed by 1% NaCl and 1 ppm $KMnO_4$ was found to be effective during severe infestation. The physical removal of the copepods and treatment of the nursery stock with 1 ppm $KMnO_4$ reduced further infestation. Infestation with *Ergasilus* was found to be highly fatal for fingerlings of *O. goramy*. However no mortality was recorded among the adults. Treatment with 2.5 ppm solution of $KMnO_4$ was found to be effective in controlling this infestation. Infestation with *Lernea* was also common among the nursery stocks causing mortality among fingerlings. Treatment of rearing tanks once in a week with 0.5% $KMnO_4$ was found to be effective in keeping the nursery stocks free from these parasites. Leeches belonging to the genus *Hemiplasia* were observed to cause heavy mortality to hatchlings in the nest.

Feeding behaviour:

While the adult of this fish is weedivorous in its feeding behaviour, the fingerlings were found to be larvivorous. When *Mansonia* larvae were given together with *Culex* larvae they consumed 46.5 and 48.5 larvae per day per gm body wt. respectively. The consumption rate observed when the larvae were given separately was 153 and 184 per gm body wt. per day. This was reduced to 69 and 85 respectively when given along with other food materials, such as plants and planktons. The study clearly indicated the larvivorous behaviour of fingerlings.

Larvivorous fishes in the control of immatures of Mansonioides:

The larvivorous efficacy of fishes such as *Macropodus*

cupanus, Ophiocephalus striatus, Tilapia mossambica, Anabas testudineus, Etroplus maculatus and *Amblypharyngodon microlepis*, which were frequently encountered in habitats free from vector breeding was subjected to laboratory investigations. Larvivorous efficacy of two exotic varieties such as *Trichogaster trichopterus* and *Osphronemus goramy* was also assessed. A comparison was made for the larval consumption of *Mansonia* and *Culex*. The number of *Mansonioides* larvae consumed per day by *T. mossambica, E. suratensis, E. maculatus, T. trichopterus* and *M. cupanus* was 512, 348, 245, 198 and 154 respectively and the number of *Culex* larvae consumed by the respective species was 456, 413, 260, 139 and 99.

An account of the fresh water fishes of Shertallai region was given earlier. The natural impact of these fishes on vector breeding was studied by conducting surveys. The larval status of the ponds was observed to be determined by some of the fish fauna in the habitat. As many as 75% of the clean ponds (n = 20) without fishes were found to support *Mansonioides* breeding whereas only 41.76% of the ponds with fishes (n = 60) were found to be positive for the larvae. The corresponding figures for polluted habitats were 95% (n = 20) and 81.25% (n = 48) respectively. From the data it is clear that the impact of fishes on vector breeding is pronounced and is higher in clean ponds rather than polluted. The proportion of negative ponds with fishes was significantly (P = 0.0061) higher than that of ponds without fishes.

The intensity of *Mansonioides* breeding in relation to the prevalence of fish species was analysed and found that the number of negative ponds with *C. orientalis* was significantly low ($P < 0.05$) compared with others viz., *M. cupanus* ($\chi^2 = 3.86$; $P = 0.04$), *A. testudineus* ($\chi^2 = 4.51$; $P = 0.03$) and *O. striatus* ($\chi^2 = 4.2$; $P = 0.04$). The analysis of *Mansonioides* breeding in relation to the presence of fishes with larvivorous behaviour shows that *M. cupanus* was present in higher proportion in polluted ponds (66.67%) that were free from larval breeding than in clean ponds (17.14%). More over, the ponds that support high vector breeding were conspicuous for the absence of this fish. Since, *T. mossambica* was the least prevalent species among larvivorous fishes, their impact on *Mansonioides* breeding could not be assessed.

When the ponds with different fish combinations were analysed for the proportion of negative ponds, it was found to be significantly ($P < 0.05$) higher with fish combinations of *M. cupanus* and *A. testudineus* when compared to the ponds without any fish species. Their impact is further evident from the fact that there were no ponds with high density of *Mansonioides* with this fish combination. Though there were few ponds of this fish combinations which were positive for larval breeding at low densities it was significantly ($P < 0.05$) lower, when compared to the ponds without any fish species.

The food habits of fishes were studied through gut content analysis of fishes collected from different habitats. Remnants of *Mansonioides* larvae (Plate III, A) were obtained in all the six species of fishes among which *Etroplus maculatus* (71.43%) had the highest followed by *M. cupanus* (58.14%) (Fig. 1.1). However, there was no significant ($P = 0.3958$) difference in the proportion of *Mansonia* larvae between these two species. But proportion of specimens of *M. cupanus* with *Mansonioides* larvae was significantly ($P = 0.028$) higher when compared with *C. orientalis* (29.41%) suggesting a higher larvivorous potential of *M. cupanus*. Food spectrum of all the fish species studied shows that the major portion of the gut contents of *M. cupanus* was larval materials of *Mansonia* (49.6%) followed by algae (26.83%). The rest of the food materials was in low quantities and ranged between 2.17% and 13.00%. While *M. cupanus* had a wide variety of food materials in their stomach, *E. maculatus* showed a very narrow range of food selectivity and the major food item was found to be *Mansonioides* larvae.

Studies on larvivorous potential:

Controlled field trials:

The efficacy of fishes such as *O. striatus, M. cupanus, A. testudinues, T. mossambica, O. goramy, T. trichopterus* and *Aplocheilus lineatus* in controlling the *Mansonioides* mosquitoes was evaluated under natural conditions in the Kadakkarappally Panchayath of Shertallai Taluk. Ponds promoting vector breeding and having similar ecological characteristics were selected for the purpose. Prior to the experiment, the weeds were removed from the ponds. The larvivorous efficacy was assessed in specially designed floating cages (60 cm X 60 cm X 60 cm) made of nylon mesh (44 mesh/inch) to avoid the immigration and emigration of both predator and prey (Plate III, B). The percentage of emergence of adults from cages

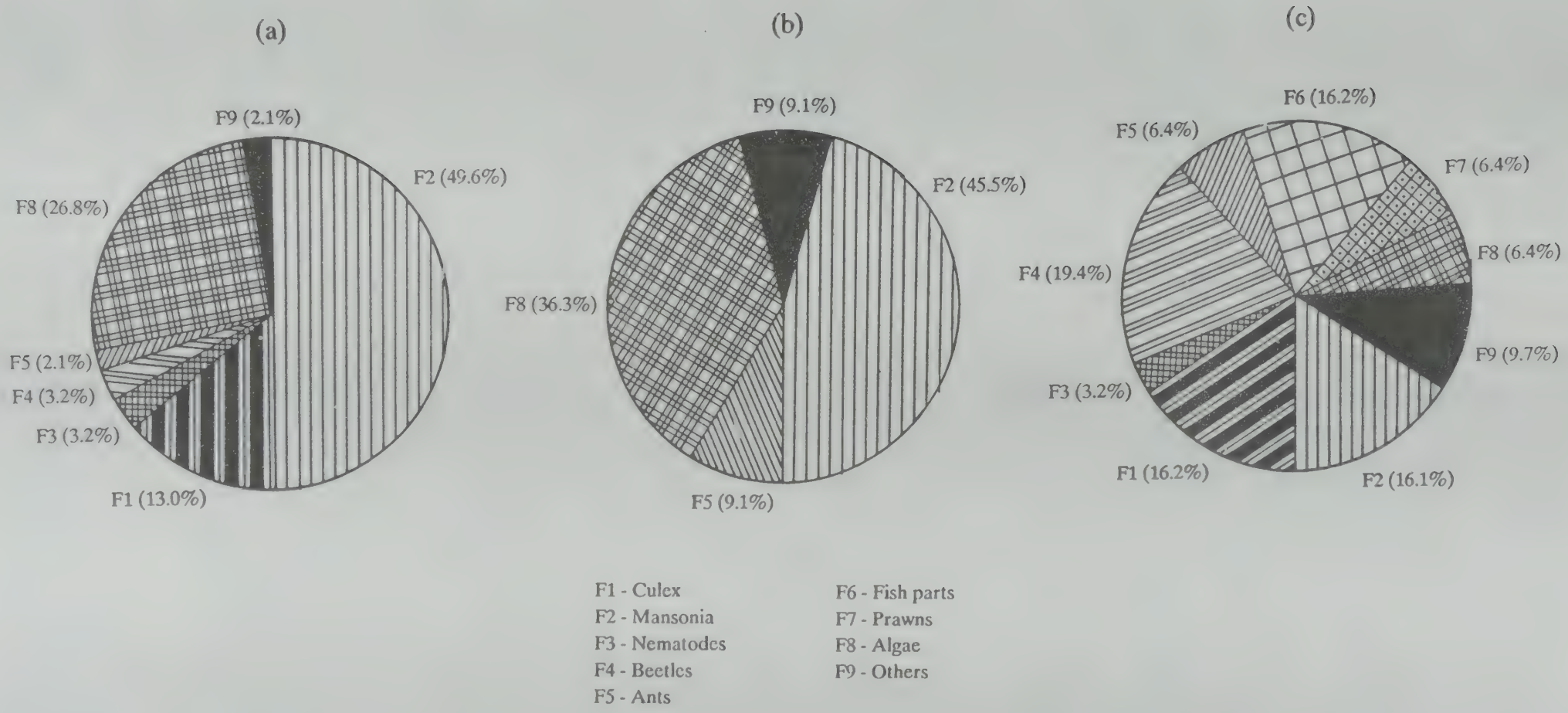


Fig. 1.1 Food spectrum of *M. cupanus* (a), *E. maculatus* (b) and *C. orientalis* (c).

without fishes was 13.40 in the polluted habitats whereas it was 7.68% in clean habitats. All the fishes except *Ap. lineatus* brought about 90% reduction in clean habitats, whereas it was over 85% in polluted habitats. The percentage emergence from control cages was significantly ($P < 0.05$) higher when compared to that of cages with fishes in both the habitats. The proportion of larvae which successfully emerged as adults in cages with fishes was significantly ($P < 0.05$) less in both clean ($F = 40.77$) and polluted ($F = 61.41$) habitats when compared to control.

The laboratory investigations as well as the controlled field trials clearly indicate *M. cupanus* as the most ideal larvivorous fish against immatures of *Mansonioides* in situations prevailing in Shertallai region. In view of the high *Mansonioides* larval control potential, studies on the biology of this species have also been undertaken.

Reproductive biology of M. cupanus:

M. cupanus is a bubble nest builder. Intensive breeding was noticed during the early monsoon season. The nest consisted of small clear space near the water edge, about 5" in diameter devoid of all grasses and weeds, except a single stalk of weed or grass in the middle, which served as an anchorage for floating egg-masses which were laid in batches and guarded by the males.

Fecundity:

The mean fecundity of *M. cupanus* ranged between 188 and 522. It varied depending on the weight of the ovary and body parameters. Though there was a significant relationship between fecundity and other parameters such as ovary weight, fish weight and fish length, the fecundity is more closely related to the ovary weight ($r = 0.7739$) when compared to the body weight ($r = 0.7336$) and total length ($r = 0.6947$) of the fish. The relationship between length and body weight is not as significant as that with fecundity and body weight which may be due to the depletion in weight followed by spawning.

Susceptibility to insecticides:

Susceptibility of *M. cupanus* to Baytex (2% W/W Fenthion, O,O-dimethyl O [3 methyl -4-(methyl thiophenyl) phosphorothioate]) which is commonly used for larval control by public health authorities was studied. Total mortality occurred when the concentration was 0.0250 mg/l and above. The LC_{50} and LC_{90} values after 24 hours of exposure were 0.0098 mg/l and 0.0081 mg/l respectively. The corresponding values after 48 hours of exposure were 0.0176 and 0.0162 mg/l. Behavioural, morphological, physiological and histopathological changes were observed under sublethal dosages.

Tolerance to physico-chemical factors:

The minimum and maximum thermal death point of *M. cupanus* was found to be 11 and 40°C respectively. The maximum efficient temperature was 37°C and the minimum active temperature 19°C. This species was observed to tolerate a salinity upto 20,000 mg/l beyond which mortality occurs. Salinity in the ponds of Shertallai region ranged from 40 mg/l to 480 mg/l during the study period and hence salinity was not found to be a detrimental factor. This fish was found to thrive well in water with BOD upto 532 mg/l which was the highest value observed in the natural habitats of this region.

1.3. COMMUNITY INVOLVEMENT AND INTERSECTORAL COLLABORATION FOR THE CONTROL OF MALAYAN FILARIASIS:

1.3.1. Filariasis Control "FILCO" Movement:

Filariasis Control (FILCO) Movement has grown into a major community force in the fight against malayan filariasis in Shertallai areas of Kerala. It has also evolved into a self-reliant body with multifaceted activities towards vector control, parasite control and dissemination of filariasis control messages through health education. While vector control through 'Shramadans' remained one of the important components of FILCO activities, Filariasis Detection and Treatment Centres (FDTC) sponsored by member organisations have screened over 12,400 people for microfilaraemia. Besides, they have also made strenuous efforts to carry health education on filariasis control to the entire nook and corner of Shertallai taluk.

During the annual meet of FILCO, they have honoured through decorations and trophies the top ranking 10 FDTC centres in terms of population coverage. Their assistance in the implementation of various fish culture schemes carried out under the aegises of NABARD, SEGP etc., was also invaluable.

1.3.2. Vector Control through Rural Development Scheme of Agriculture Department:

The Kerala State Agriculture Department has recently drawn out a novel scheme, "Self Employment Generation Programme (SEGP)" with the objective of creating employment for the unemployed rural youth through land development activities. The land development activities in Shertallai taluk were primarily restricted to pond renovation. All the village level Agricultural Offices ("Krishi Bhavans") were entrusted with the responsibility of identifying the beneficiaries. Monetary assistance was arranged to the beneficiaries through different financial institutions (Nationalised banks) with 25% subsidy from the State Government.

The VCRC has entered into a collaborative venture with Agriculture Department, utilising the above captioned scheme towards the disease vector control. The idea of 'Composite fish culture' in all the ponds, deweeded and renovated under the SEG Programme was well conceived by both the implementing agency i.e. the State Agriculture Department and the recipient farmers. The VCRC has ensured the beneficiaries, the availability of fish fingerlings on subsidised rates, besides the necessary technical guidance. In this process, as many as 1,611 ponds were made free from weeds which were once supporting the breeding of *Mansonioides* mosquitoes, the vectors of malayan filariasis.

1.3.3. Composite fish culture scheme through NABARD:

Many administrative bottlenecks faced by the bank institutions in the proper implementation of the scheme were identified by the VCRC and sorted out through joint high level meetings of NABARD, Bankers and VCRC. As a result of this the NABARD held discussions with various Bankers at different levels, and suggested them to use their own discretions, wherever possible to waive many formalities which impede the scheme. Yet, it was possible to utilise only 198 units (ponds) during this year. The Union Bank of India alone has achieved the physical target assigned. To improve the situations, the Registrar of Kerala State Co-operative Agriculture Development Bank has come one step forward and issued a notification to the Shertallai Branch to simplify the norms in sanctioning the loans. This development is expected to bear fruits in a big way in the coming financial year, and the signs are already visible in the current year as a large number of applications have already been processed in the light of this notification.

1.3.4. A collaborative venture for Vector Control with the Agriculture Departmental Agencies:

It has already been reported in the previous report that the utility of "sunhemp" as an alternative manure source was explored through the 'Sunhemp Village Project' at Ponnittussery. This project has drawn the attention of the Agricultural Experts of Kerala Government and they have come forward with an elaborate scheme to extend this programme to the entire taluk. It has also been agreed upon that under this scheme the sunhemp seeds will be made available to the community free of cost through the subsidies of Agriculture Department, Government of Kerala and VCRC. The Officers of "Krishi Bhavan" will be the implementing authorities of this programme and they will ensure that every farmer enjoying this benefit will keep his environs free from aquatic weeds.

1.3.5. Filariasis Control activities by Students' Community:

The Students' Filariasis Control Club (SFC club) has given more emphasis during the year, on the detection and elimination of parasite load from their fellow-mates. Through such clubs, as many as 3,041 students were screened for filariasis through night blood smear examination. Mass drug administration with low dose DEC "By the students For the students" has been conducted in 14 schools of the taluk, covering 10,622 students.

A horizontal level of health education was also imparted by the volunteers of SFC clubs in 16 high schools. In each of these schools, one of the SFC club members delivered health education talk on filariasis during the regular school assembly where the entire student community of the respective school is gathered.

Being motivated by the activities of SFC clubs, the National Cadet Corps (NCC) of the schools and colleges (9 schools and 3 colleges) of Shertallai taluk also extended their services to fight against filariasis. Accordingly, the Commanding Officer of 11 Kerala Bat-

talion (Alleppey) has issued instructions to all the concerned NCC Officers to get their Cadets actively associated with the disease vector control activities of VCRC.

The NCC Cadets, during the parade days, regularly devote some hours for deweeding of ponds through 'Shramadans'. In this process, a large number of water bodies accounting for an area of 1,44,800 sq.m. were made weed free by the Cadets from seven schools and 2 colleges.

The Cadets have also decided to adopt a village for demonstrating and coordinating the various activities of filariasis control. Towards this, selected Cadets (70 numbers) and Officers (12 numbers) have been provided with training on night blood smear collection. Through them 414 children who have come from different schools to participate in youth festival held at Shertallai were screened for filariasis.

It is a matter of pride for both the student community as well as the teachers, as one of the SFC club volunteers bagged a prestigious National awards and a NSS Programme Officer a State award for their meritorious social service which included grandiose activities of filariasis control.

1.4. PILOT PROJECT ON DEVELOPMENT OF INTEGRATED VECTOR CONTROL AT THE VILLAGE LEVEL BY COMMUNITY:

A project on the development of integrated vector control at village level by the community was initiated as an offshoot of Shertallai project in the adjoining village Mararikulam South, one of the highly endemic pockets for Malayan Filariasis in Alleppey District, with the support of WHO. While the community was prepared to shoulder the entire responsibility of the disease vector control operations, the VCRC was committed only to render the necessary technical guidance with software and suitable control agents. The VCRC is also monitoring the programme.

Since the project is time bound and target oriented, it was restricted to Ward Nos. I to V of the village, with a population of 15,600 spread over an area of 7 sq.km. Adjoining to this area, a Barrier Zone (Ward No. VI) and a Check Zone (Ward Nos. VII and IX) were also maintained.

Necessary base-line data on both entomological and clinico-parasitological aspects were collected. A year long programme was prepared (Fig. 1.2). To begin with, identification and training of local leaders was found to be a prerequisite in view of the socio-political background of the study area. The elected representatives of the local bodies at Panchayath and Ward level were approached and were apprised of the objectives of the project, to ensure their cooperation, right from the beginning. With their assistance a core group of volunteers representing a cross section of the target community was selected.

The operational area (Ward Nos. I-V) was divided into 36 blocks, each with an average of 100 households. For each block, 60 volunteers were selected by the community and were grouped, each group consisting of 15 volunteers. Each group was assigned independent responsibilities for source reduction/fish culture, spray operations, parasitological screening and mass drug administration. There were at least three volunteers for every 5 houses. The Kerala State Programme for Total Literacy Drive, was best utilised by combining their activities with disease vector control.

Action programmes were designed to carry out, block-wise. Intensive health education campaigns were made with volunteers, who in turn pass on the message to the recipient community. The volunteers were imparted with the necessary technical knowledge, on all aspects of filariasis control to enable them to undertake the whole operation, at the grass root level.

Detection of microfilaria carriers and selective treatment of the positive cases were given prime importance, besides mass drug administration (MDA) with Diethylcarbamazine (DEC) and weed/vector control. Volunteers from 30 blocks were given training. Night blood survey and single course MDA (DEC 6mg/kg body wt. X 12 days) have been carried out in 20 such blocks by the volunteers. As many as 223 microfilaraemic patients were detected out of 8,807 screened, and all of them were given selective chemotherapy. Over 6,100 people were also covered under mass drug therapy, exclusively through community efforts.

Control of adult vector population was also given due importance simultaneously with other control measures. The indoor residual treatment with Deltamethrin (application rate : 25 mg a.i /m^2) was car-

PILOT PROJECT ON

DEVELOPMENT OF INTEGRATED VECTOR CONTROL

AT THE VILLAGE LEVEL BY COMMUNITY

PROJECT PLAN

PROJECT AREA: MARARIKULAM SOUTH PANCHAYATH

WARD NOS: I-V

POPULATION: 15,600

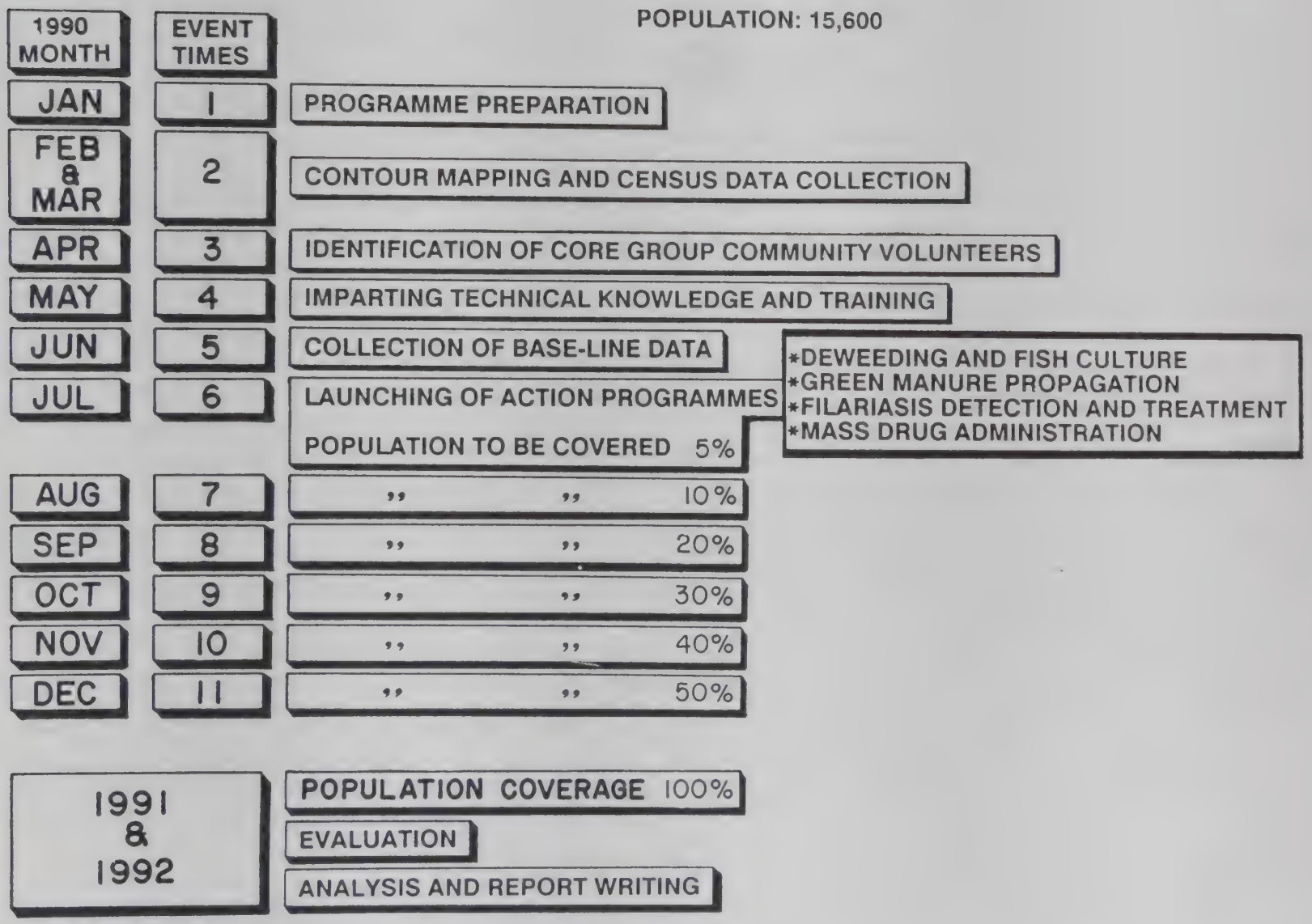


Fig. 1.2

ried out against adult vector mosquitoes. The spray operations were done with the total support and involvement of the community. The community was prepared for spray operation through street corner meetings in various localities of the study area and through personal contacts made by community volunteers. The acceptance of the community was obtained after clearing their doubts through such meetings. Each spray team consisted of four community volunteers, one for spraying and rest for rendering liaison work. They have also helped in the preparation of the houses for spraying by removing the food/eatables outdoors, and covering other items which are to be protected. Care was taken in selecting healthy volunteers as spraymen and they were given training in spraying and handling of the compression sprayers, and also the precautionary measures to be followed. The spraymen were provided with protective clothing (Overalls) during spray operation. A drastic reduction in the indoor resting density of *Mansonioides* mosquitoes was achieved and the residual effect lasted for a period of three months.

1.5. EPIDEMIOLOGICAL STUDIES:

1.5.1. Interim evaluation of impact of various control measures:

Epidemiological evaluation which included parasitological and clinical examination of the population and entomological surveys was carried out three years after the initiation of control programmes in Kadakarapalli and Shertallai South Panchayat which were under vector management and mass drug therapy respectively. Concurrent assessment was also carried out in Mararikulam South Panchayat where there was no intervention except selective therapy of microfilaria carriers detected in sample surveys for ethical reasons.

Composite fish culture involving fast growing edible fish varieties such as *Catla catla, Labeo rohita, Cirrhana mrigala, Cyprinus carpio, Labeo fimbriatus* and weed eating grass carp, *Ctenopharyngodon idella* was introduced in 1986 to control aquatic weeds and thereby breeding of *Mansonioides* mosquitoes. Kadakarapalli village, with a population of 16,165 (1981 census) was selected for assessing the fish culture programme in the control of vectors. A total of 2,065 ponds enumerated in this area of which 1,467 ponds were found fit for fish culture as the rest were polluted due to husk retting. All the suitable ponds were stocked with fish fingerlings of the above varieties at the rate of 25/100m^2. All the ponds including polluted ones were deweeded initially and the suitable ponds were prepared for fish culture by the community. Removal of weeds in the polluted ponds was also done by the community as and when the weeds started reappearing. All these ponds were restocked with fish fingerlings following their harvest after 10 to 12 months.

Two areas in Shertallai South Panchayat, with population of 22,681 in 5,639 households and 6,192 in 1,749 households were selected for evaluating the effectiveness of mass single dose DEC treatment (6 mg/kg body wt.) given annually and biannually respectively. Pre school children were covered at 'anganwadi centres' while school children at schools. House visits were also made to cover the adult population. These visits were made between 19.00 and 22.00 hours in order to cover all the family members who were expected to be at home during these hours. Infants and pregnant women were not included. Dosage was decided based on body weight, and, on the spot intake of drug was ensured by the team. A team including a physician visited all the households/ schools on the following day to monitor and treat side reactions, if any. First round of annual and biannual treatment was initiated in the month of April 1987, second biannual in the month of October 1987, third round of biannual and second round of annual in the month of April 1988 and fourth round of biannual in the month of October 1988. All the rounds were completed within 2 to 3 months from the initiation of treatment.

A check area with a population of 49,836 was maintained without any intervention and this area is contiguous as well as ecologically and epidemiologically comparable. A barrier zone was also maintained to reduce the chances of infiltration of mosquitoes to and from other areas under intervention.

Pre control parasitological and clinical surveys were made with a minimum target coverage of 10 % and 2 % of the population for parasitological and clinical examination respectively, in all the three areas. These surveys were repeated after six months of the completion of last round of mass treatment as recommended by WHO (WHO, 1974). A cohort of population surveyed in 1986 (pre control) was also

resurveyed in 1989 (post control). Entomological assessment was made by collecting indoor resting mosquitoes from 15 catching stations and man biting mosquitoes through all night man biting collections from one fixed station, at monthly intervals in each study area.

The population covered under annual single dose mass treatment was 13,982 (62.11%) in the first round and 13,278 (58.99%) in the second round. In the biannual mass treatment the coverage was 2,909 (47.29%), 2,542 (41.33%), 3,232 (52.54%) and 4,060 (66.01%) respectively in first, second, third and fourth rounds. The coverage was minimum in the age class 0-9 years (27.94% to 49.10%) and was maximum in the age class 10-19 years (57.54% to 98.18%). The proportion of people showing side reactions ranged from 6.21% to 8.4% in different rounds of mass treatment. Headache and fever were the predominant reaction (62.8% of the total cases developed side reactions).

The age specific prevalence of microfilariae (mF) in the pre and post control surveys in vector control, annual mass treatment, biannual mass treatment and check areas are shown in figure 1.3. Age specific analysis on mF prevalence showed that in the precontrol surveys the lowest mF prevalence was seen in the age class 0-9 years and the maximum in 10-19 years in all the study areas. There was a reduction in the overall mF prevalence in all the study areas and it was minimum in the check area. While the reduction in proportion of mF carriers was significant in all the three control areas it was not significant in check area (Table 1.3). The age specific pattern of pre and post control mF prevalence showed that over 50 % reduction in mF prevalence was achieved in all the age classes in all the three control areas and it was less than 50 % in check area. Increase in mF prevalence in the age class 0-9 years in the check area, though not significant, revealed the higher rate of acquisition of new cases. When the reduction in mF prevalence was compared using Log Odds ratio, maximum reduction was seen in biannual mass treatment area (Table 1.3) which was significantly ($Z = 6.99$; $P < 0.05$) higher than in the annual mass treatment area. When compared to vector control area the reduction in annual mass treatment area was significantly ($Z = 18.31$; $P < 0.05$) higher. However, the proportion of microfilaraemia cases was significantly ($Z = 102.86$; $P < 0.05$) brought down in vector control area when compared with that of check area.

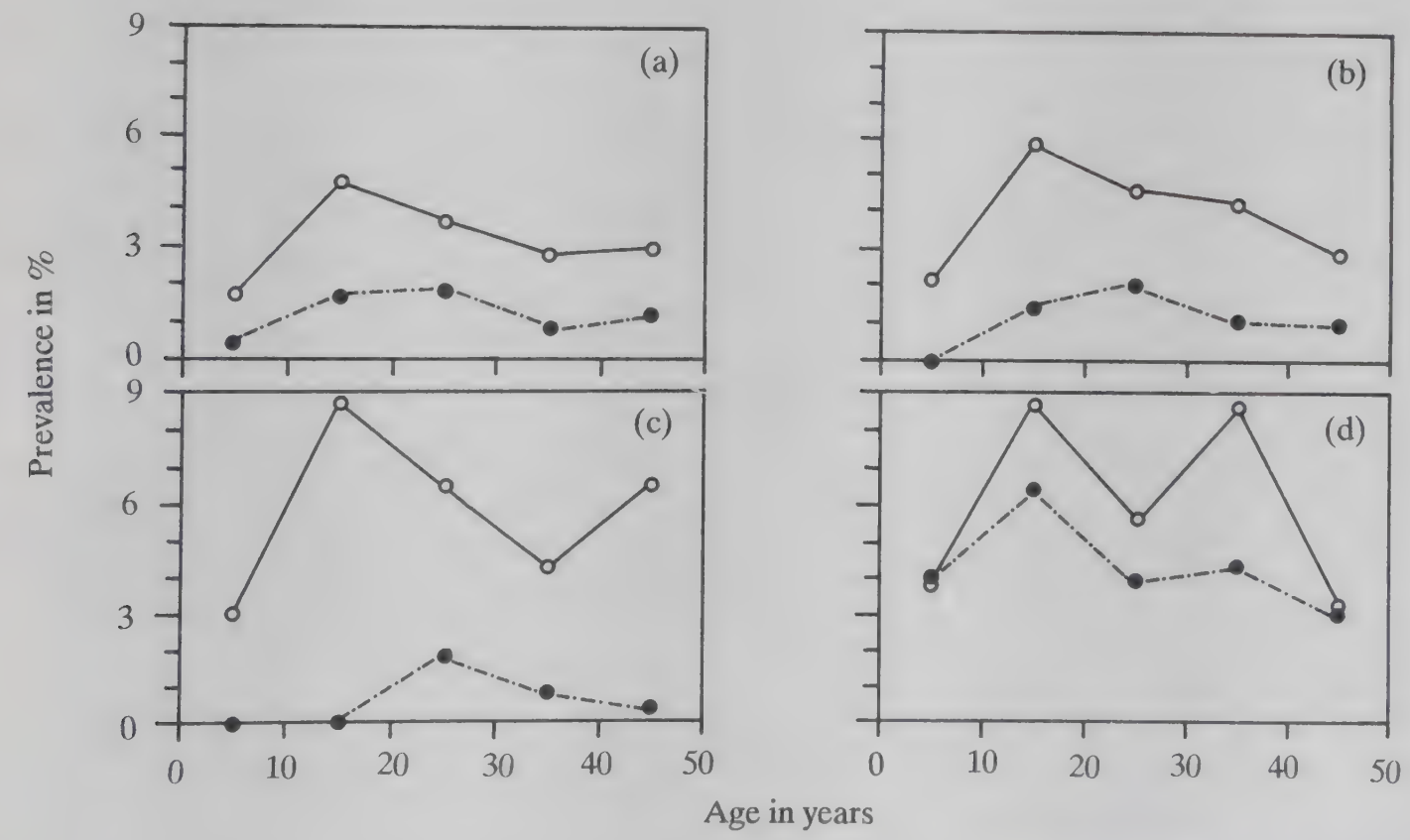


Fig. 1.3 **Microfilaria prevalence in pre (○) and post (●) control surveys in vector control (a), annual mass treatment (b), biannual mass treatment (c) and check area (d).**

Table 1.3.

Age specific Microfilaria prevalence and intensity in the study population.

Area	Age class	mF prevalence in %			Proportion significance P value	mF intensity*		
		Pre-control	Post-control	% change		Pre-control	Post-control	% change
Vector control								
	0-9	1.75	0.47	-73.29	0.0515	0.624	0.019	-97.00
	10-19	4.75	1.67	-64.78	<0.05**	0.892	0.132	-85.21
	20-29	3.67	1.82	-50.37	0.0671	0.522	0.176	-66.25
	30-39	2.83	0.81	-71.27	<0.05**	0.220	0.037	-83.38
	>=40	2.97	1.18	-60.17	<0.05**	0.477	0.130	-72.86
	Total	3.36	1.27	-62.16	<0.05**	0.582	0.111	-80.94
Annual mass treatment								
	0-9	2.20	0.00	-100.00	<0.05**	0.148	0.000	-100.00
	10-19	5.93	1.49	-74.95	<0.05**	1.087	0.200	-81.64
	20-29	4.57	2.11	-53.95	<0.05**	0.865	0.147	-82.96
	30-39	4.27	1.14	-73.32	<0.05**	0.357	0.054	-84.83
	>=40	2.92	1.06	-63.88	<0.05**	0.239	0.125	-47.56
	Total	4.02	1.23	-69.39	<0.05**	0.560	0.120	-78.56
Biannual mass treatment								
	0-9	3.08	0.00	-100.00	0.0917	0.492	0.000	-100.00
	10-19	8.73	0.00	-100.00	<0.05**	1.341	0.000	-100.00
	20-29	6.52	1.86	-71.43	<0.05**	0.609	0.050	-91.84
	30-39	4.32	0.82	-81.01	0.083	0.374	0.033	-91.24
	>=40	6.55	0.40	-93.96	<0.05**	1.062	0.008	-99.26
	Total	6.27	0.62	-90.17	<0.05**	0.864	0.017	-98.00
Check Zone								
	0-9	3.85	4.08	+06.03	0.645	0.115	0.931	+706.74
	10-19	8.72	6.48	-25.79	0.408	1.966	1.775	-9.76
	20-29	5.66	4.04	-28.69	0.602	1.594	1.087	-31.81
	30-39	8.70	4.47	-48.61	0.137	1.098	1.482	+35.02
	>=40	3.39	3.10	-8.46	0.974	0.616	0.579	-6.05
	Total	6.08	4.41	-27.51	0.094	1.177	1.148	-2.44

* : Mean mF count of the population
** : Significantly different
\- : Decrease
\+ : Increase

The age specific pattern of mean mF count (mfc) per individual in the pre and post control surveys in the study areas is given in figure 1.4. A total of 553 cases in vector control area, 814 in annual mass treatment area, 590 in biannual treatment area and 244 in check area were longitudinally followed after a period of three years. The mean mfc per individual in the pre and post control surveys was significantly brought down from 0.99 to 0.01 ($T = 3.83$; $P < 0.05$), from 0.80 to 0.07 ($T = 2.86$; $P = 0.004$) in biannual and annual mass treatment areas respectively. In the vector control area also there was reduction in the mean mfc per individual from 0.78 to 0.17, but it was at its statistical limit ($T = 1.91$; $P = 0.057$). In the check area though there was reduction in mean mfc in the post control survey (1.91 to 1.07) it was not significant ($T = 1.38$; $P = 0.17$).

The average annual incidence of new microfilaria cases in the age class 1-7 years per 1000 population was calculated to be 2.86, 4.86, 9.18 and 2.38 in the precontrol surveys in vector control, annual mass treatment, biannual mass treatment and check areas respectively. There was reduction in the incidence of microfilaraemia in the vector control area (1.43) and no new cases were detected in both annual and biannual mass treatment areas in the age class 1-7 in the post control survey suggesting that the incidence of new cases was practically nil. However, in the check area the incidence increased from 2.38 to 28.44.

The rate of acquisition of new infections per individual per annum, calculated from the longitudinal follow-up of a cohort population with both mF positive and negative cases was observed to be the lowest in biannual mass treatment area (0.0023) which was followed by vector control area (0.0038) and annual mass treatment area (0.0122). In the check area however, it was as high as 0.0133. When the proportion of amicrofilaraemic individuals becoming microfilaraemic in the control areas was compared with that of check area, it was significantly lower in biannual mass treatment area ($P = 0.009$), whereas it was at its statistical limit in vector control area ($P = 0.055$) and the difference was not significant in annual mass treatment area ($P = 0.144$). The rate of loss of infection per case per annum was recorded to be the highest in biannual mass treatment area (0.325) which was significantly ($P = 0.038$) different from that of check area (0.241). Though the rate of loss of infection was relatively higher in the annual mass treatment (0.323) and vector control areas

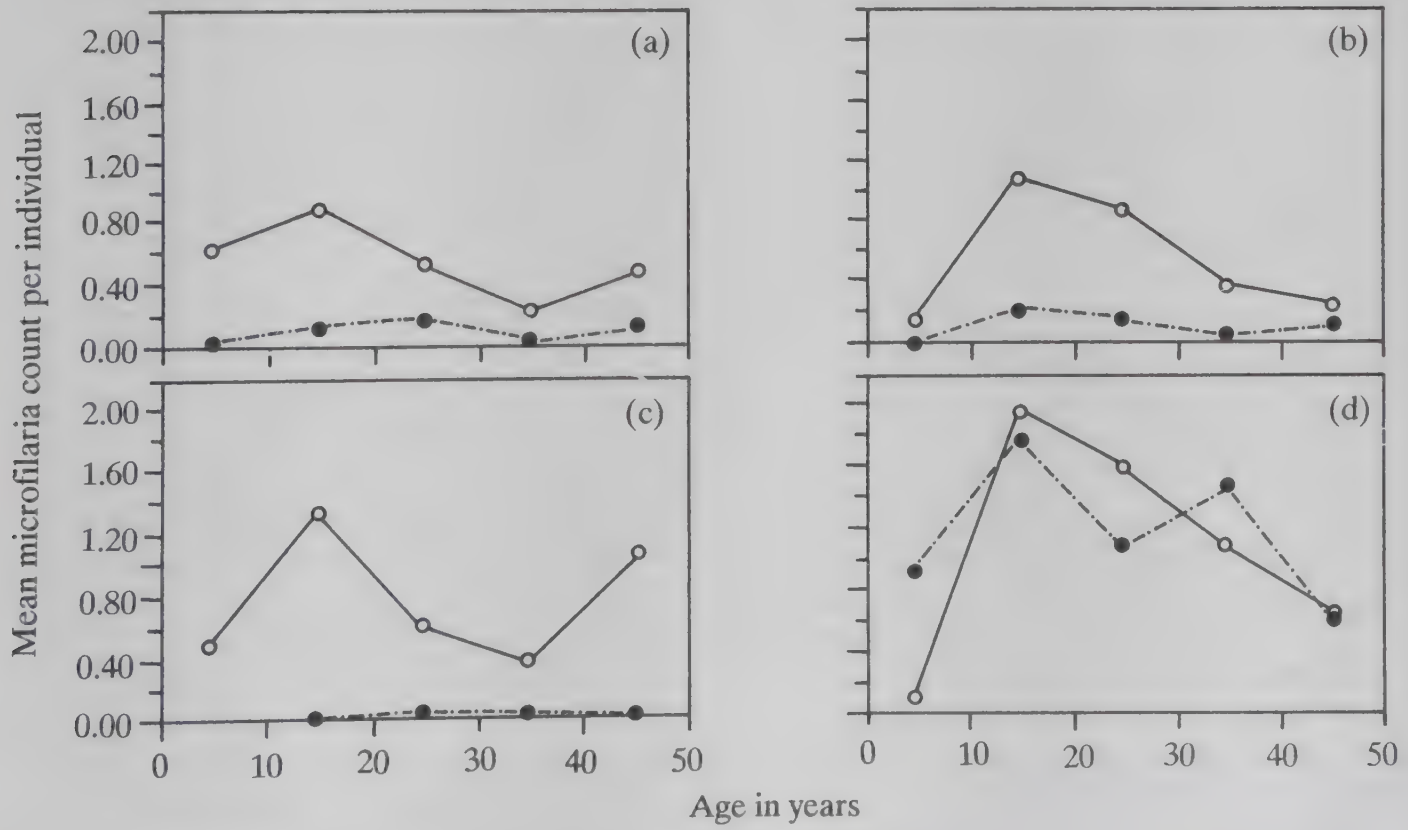


Fig. 1.4 **Mean microfilaria count in pre (○) and post (●) control surveys in vector control (a), annual mass treatment (b), biannual mass treatment (c) and check area (d).**

(0.269) they were not significantly ($P > 0.05$) different from that of check area. The rate of acquisition in the vector control area was nearly six times lesser than that of check area, reflecting the reduction in transmission following vector control measures. Relatively a higher loss rate of infection recorded both in annual and biannual mass treatment areas indicates the effect of chemotherapy in reducing the parasite load. The effect of biannual mass treatment was comparatively more as there was significant reduction in both acquisition and loss of infection when compared to check area.

The pre control infectivity index of the population in the vector control, annual mass treatment, biannual mass treatment and check area was 1.71, 2.1, 2.7 and 3.72 and was reduced to 0.56 (67.25%), 0.50 (76.19%), 0.25 (90.74%) and 2.18 (41.4%) respectively in the post control survey. Though there was natural decline in the infectivity index of the population in the check area, as high as 65% reduction was achieved in areas under vector control and parasite control.

The changes in the prevalence of acute manifestations, recent oedema, persistent oedema with or without elephantoid changes and chronic cases associated with or without acute manifestations in the study population is given in table 1.4. The prevalence of acute manifestations though reduced in all the areas including check area, the reduction in the proportion was significant only in annual mass treatment and vector control areas, whereas it was not statistically different in biannual mass treatment and check areas. The prevalence of recent oedema showed a significant reduction in the post control surveys in all the control areas whereas in the check area the reduction in the post control was not significant. When the change in the prevalence of recent oedema cases in the post control survey was compared between different study areas, it was found that the reduction was significantly higher in biannual area ($Z = 9.38$; $P < 0.05$) than that of annual mass treatment which recorded significantly ($Z = 2.53$; $P < 0.05$) higher reduction than that of vector control area. However, in the vector control area it was significantly ($Z = 6.99$; $P < 0.05$) higher than that of check area. Persistent oedema with or without skin changes did not differ significantly from its pre control survey in all the study areas, though the prevalence was observed to be reduced in the post control survey. The analysis on the proportion of lymphoedema chronic cases associated with or without acute manifestations such as filarial fever, lymphangitis and lymphadenitis showed that over 60% reduction in the cases associated with acute episodes was significant in all the three areas after the implementation of control measures, whereas it was only 24.8% and not significant in the check area. When this reduction was compared between different study areas, it showed that in biannual mass treatment area it was not significantly ($Z = 0.118$; $P > 0.05$) different from that of annual mass treatment area. However, when compared to vector control area the reduction was significantly higher in both annual ($Z = 6.7$; $P < 0.05$) and biannual ($Z = 8.8$; $P < 0.05$) mass treatment areas, indicating the beneficial effects of mass therapy in reducing the morbidity of diseased individuals. Age specific analysis showed that the reduction in morbidity in chronic lymphoedema cases was significant ($P < 0.05$) in the age class $>= 40$ years.

All the three species of *Mansonioides* viz., *Mansonia annulifera, M. uniformis* and *M. indiana* were observed to be infected and infective with *B. malayi* larvae and hence all the three species were considered together for analysing density and infection. The per man hour density of indoor resting vector mosquitoes ranged from 0.25 to 4.90 in different months during the period prior to the introduction of vector control measures (Fig. 1.5), the mean density being 2.49.

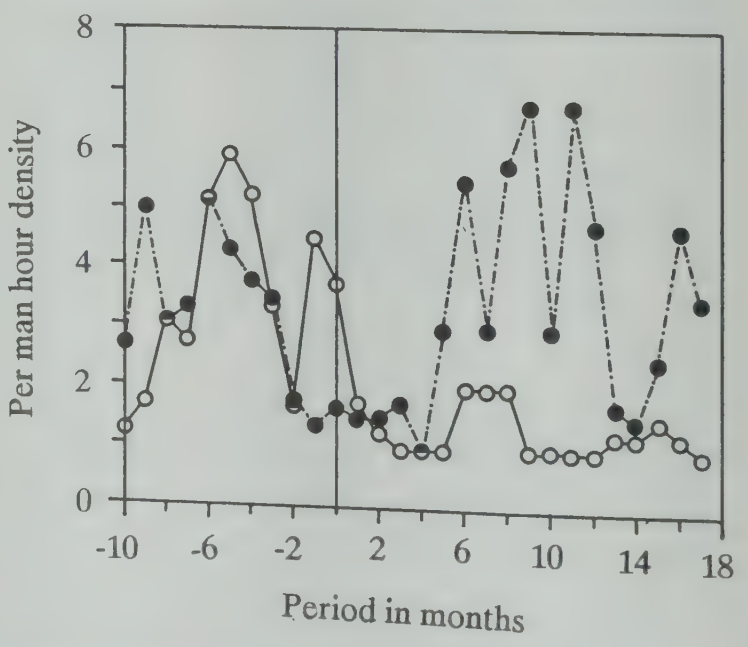


Fig. 1.5 Relative density of indoor resting vector mosquitoes in vector control (○) and check area (●) prior to and after initiation of vector control programme.

Table 1.4.

Change in the prevalence of clinical manifestations in the study population.

Area	Type of manifestation	Prevalence in %			Proportion significance P value
		Pre-control	Post control	% change	
Vector control		(n = 639)	(n = 671)		
	1. Filarial fever/ adenolymphangitis	5.48	1.64	70.07	0.0002*
	2. R O	1.41	0.30	78.72	0.0251*
	3. P O & Ele	11.41	9.39	17.70	0.2642
	4. Chronic + acute	10.17	3.73	63.32	<0.05*
Annual mass treatment		(n=751)	(n=807)		
	1. Filarial fever/ adenolymphangitis	5.86	0.62	89.42	<0.05*
	2. R O	1.86	0.12	93.55	0.0002*
	3. P O & Ele	9.64	7.56	21.58	0.239
	4. Chronic + acute	9.59	2.85	70.27	<0.05*
Biannual mass treatment		(n = 443)	(n = 500)		
	1. Filarial fever/ adenolymphangitis	3.39	1.80	46.90	0.740
	2. R O	2.26	0.20	91.15	0.0032*
	3. P O & Ele	11.29	9.40	16.74	0.398
	4. Chronic + acute	11.74	3.80	67.63	<0.05*
Check area		(n = 155)	(n = 825)		
	1. Filarial fever/ adenolymphangitis	8.39	4.97	40.76	0.091
	2. R O	1.29	0.61	52.71	0.289
	3. P O & Ele	16.13	11.88	26.35	0.182
	4. Chronic + acute	9.67	7.27	24.82	0.906

R O : Recent Oedema
P O & Ele : Persistent Oedema and Elephantiasis
* : Significantly different.

Fortnightly monitoring of resting vector population, carried out for 17 months following fish culture programme showed that the per man hour density fluctuated between 0 and 1.0, (mean = 0.40) and this was significantly (T = 4.59; P < 0.05) lower than pre control level. The man biting rate of vector mosquitoes (No. per man per night) fluctuated between 11 and 61 during the pre control period (mean = 36.5). During the post control period of vector control it ranged from 0 to 36, with a mean of 10.23 which was significantly (T = 3.11; P < 0.05) lower when compared to pre control level. In the check area the indoor resting vector density fluctuated from 0.67 to 4.07 (mean = 2.2) and 0.5 to 5.75 (mean = 2.42) for the periods corresponding to pre and post vector control. The man biting rate during the respective periods was 9 to 36 (mean = 27) and 6 to 46 (mean = 32), indicating a natural increase in vector population. In the biannual mass treatment area also there was no significant (P > 0.05) change in both indoor resting and man biting density.

The percentage of vector mosquitoes infected with filarial larva was brought down from 2.05% to 0.77% after mass treatment and the proportion of infected mosquitoes was significantly (P < 0.05) lower in the post control period when compared to that of pre control period. The percentage of infective mosquitoes was also brought down to 0.14 from its pre control level of 0.29. Though over 50% reduction in infectivity was achieved, it was not significantly (P > 0.05) different. In the check area the percentage of vector mosquitoes infected was 1.57 and 3.57 during the corresponding pre and post control periods of mass treatment. The infectivity rate for the respective periods was 0.26 and 0.54. Thus there was over twofold increase in both vector infection and infectivity in the check area. While infection in vector mosquitoes was noticed in most of the months during the period of observation in the check area, vector infection was noticed only prior to and during the implementation of mass treatment. None of the mosquitoes collected during the period of 14 months following the final round of chemotherapy was found infected. In the vector control area both infection and infectivity rates were very low during the pre control assessment and this did not permit to make any meaningful comparison between the pre and post control periods.

The intensity of infection (No. filarial larva of any stage/mosquito dissected) in the vector population for different months of observation in both biannual mass treatment and check area is shown in figure 1.6.

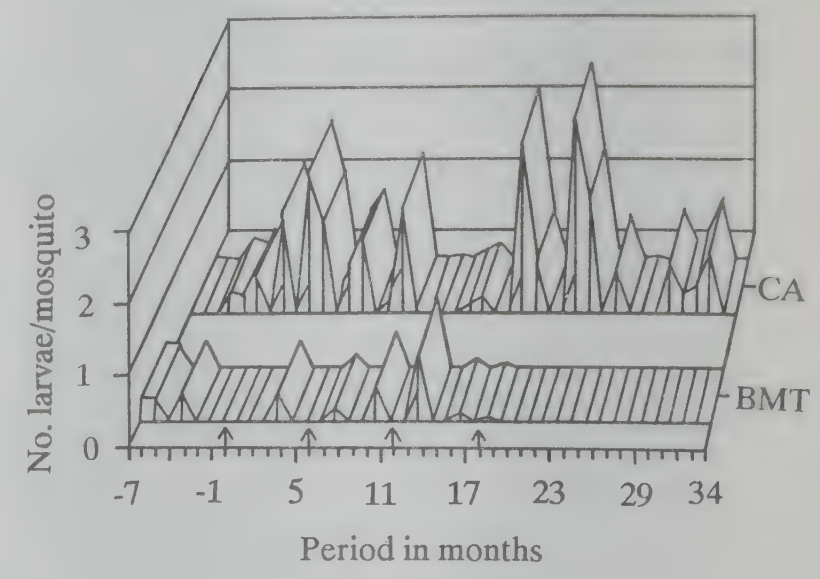


Fig. 1.6 Intensity of infection in vector population in biannual mass treatment (BMT) and check area (CA), prior to, during and after implementation of mass treatment.

↑ Indicates the initiation of different rounds of mass treatment.

There was a decrease in the mean number of filarial larva from 0.184 to 0.087 after the initiation of biannual mass treatment, whereas in the check area it showed an increase from 0.191 to 0.521 during the corresponding periods of observation. However, the number of infective larva per mosquito did not show any change from its pre control level (0.002) in the mass treatment area while in the check area it increased from 0.034 to 0.047.

Thus interruption in transmission was evident from the significant reduction in vector density (72-83.9%) and proportion of infected vector mosquitoes (62.2%) following the implementation of vector control and parasite control measures respectively. Both vector control and mass treatment were found to be feasible as well as acceptable by the community as the former was linked with income generating scheme and the latter reduced morbidity. However, it is important that parasite control should be augmented with vector control so as to achieve the goal of eliminating the foci of transmission within a time frame.

1.5.2. The dynamics of microfilaraemia and its relation with development of disease in *B. malayi* infection:

Age specific estimates of the rates of gain and loss of *B. malayi* microfilaraemia based on longitudinal observations have been made, using the reversible catalytic model. These estimations enable to calculate the proportion of people who had been microfilaraemic but have subsequently become amicrofilaraemic. This population is assumed to be at the risk of developing disease manifestations as was observed in bancroftian filariasis. This estimated proportion at risk in different age classes is compared with the observed age-prevalence of malayan filariasis disease in Shertallai endemic population.

A total of 2,275 persons was examined longitudinally for mF both in 1986 and 1989. Of these 257 individuals were found to be microfilaraemic in 1986. Among the 257 mF carriers of 1986, reexamined in 1989, 214 were treated and only 43 cases did not take any kind of chemotherapy. Out of these 43 cases 25 were shown to be amicrofilaraemic in 1989, accounting for a natural loss of microfilaraemia of 0.5814. Under the assumption that in a population where transmission was interrupted by various control measures, the instantaneous rate of loss of microfilaraemia (β) and fecundic life span of adult parasite were found to be 0.2903 and 3.44 yrs respectively. The rate of loss of microfilaraemia was independent of gender ($\chi^2 = 0.016$; P = 0.899). The logit regression of 'β' against host age indicated that the rate of loss of microfilaraemia is independent of host age also ($\chi^2 = 0.015$; P = 0.9025).

Out of 2,018 amicrofilaraemic individuals who were examined longitudinally in 1989, 16 had become infected accounting for a rate of gain of 0.79%. The rate of gain was independent of gender ($\chi^2 = 1.05$; P = 0.3052). The polynomial regression of 'm' indicated that the rate of acquisition is dependent on host age (F = 436; d.f = 2,2; P = 0.0023).

Since the amicrofilaraemic population included a proportion of individuals who were microfilaraemic in the past but subsequently became mF negative during the progression to disease, these individuals were eliminated from the presumed uninfected population using the disease status among amicrofilaraemic persons. Thus by considering the actual uninfected population who were susceptible for infection the corrected rate of gain of microfilaraemia was estimated (Table 1.5).

Table 1.5.

Age specific rate of acquisition of infection (crude and corrected) with respect to disease status in amicrofilaraemic individuals.

Age gr. (Years)	mF -ve in 1986	Proportion gaining infection 1989	Disease status in mF -ve individuals in 1986		Corrected proportion gaining infection
			sampled	Disease rate (proportion)	
0-9	314	0.0032	608	0.0066	0.00321
10-19	506	0.0099	791	0.0392	0.01028
20-29	304	0.0132	679	0.0781	0.01427
30-39	297	0.0139	547	0.1225	0.01588
> =40	607	0.0033	1259	0.2240	0.00425
Total	2018	0.0079	3884	0.1125	0.00893

The linear regression analysis indicated that the risk (of developing disease) factor is age dependent ($F = 35.38$; $P = 0.0082$). The observed age specific prevalence of disease was linearly related to the estimate of age specific risk ($r = 0.9609$; $P < 0.05$). On an average, 88% of population at risk developed manifestations of disease. Age-prevalence of filariasis disease was linearly related to the age-prevalence of lymphoedema ($r = 0.9977$; $P = 0.0001$). For all age classes on an average 95.51% of cases of diseased persons had lymphoedema.

Lymphoedema was classified into recent oedema (grade I) and chronic oedema (grade II and III). The age specific prevalence of these two grades of oedema was compared with that of proportion at risk of developing lymphoedema. Linear regression analysis indicated that on average 13% and 75% of population at risk developed recent oedema and chronic oedema respectively. It was observed that while the chronic oedema prevalence showed a monotonic age dependent rise, recent oedema was more or less uniform beyond 15 years of age.

The age specific estimated 'risk factor' in the same area was compared with the corresponding disease prevalence which ranged from 2.7% (1-5yrs) to 45.5% (>5yrs). The significant linear relationship ($r = 0.9341$; $P < 0.05$) between the age specific risk factor and the observed disease prevalence showed that about 46% of population at risk developed disease manifestations.

The present analyses showed that the age specific predicted prevalence of mF, based on corrected rate of acquisition and the rate of loss of microfilaraemia closely mirrored the observed prevalence. The general decline in the predicted values may either be due to higher rate of loss of microfilaraemia or the decline in the rate of acquisition. Further, these findings are in agreement with the hypothesis that mF are produced following the infection and destroyed by the host immune response and a proportion of them develop clinical symptoms. However, in older age classes (20-39 years) the proportion predicted to be at risk was more than the disease prevalence. This over estimate may either be due to disappearance of acute symptoms spontaneously or the long duration required to develop chronic manifestations.

1.5.3. **Health Care Delivery:**

Filariasis clinic:

Filariasis clinic at VCRC Field Station continued to function on all Wednesdays ((18.00 to 23.00 hrs) and Saturdays (08.30 to 13.00 hrs). A total of 2,574 new patients registered during the period under report and on examination 1,485 (57.69%) persons were clinically diagnosed as filarial patients. Including the old patients who reported for follow-up treatment, as many as 7,512 patients were treated for filariasis with DEC and other supportive measures. Out of 3,295 smears collected in the field station where the facilities were made available for blood examination on all the days between 20.00 hrs and 22.00 hrs, 25 (0.76%) were shown to be positive for *B. malayi* and were treated with DEC.

Filariasis Detection Camps:

As many as 19 community voluntary organisations were involved in arranging night camps to screen people for microfilaraemia. A total of 5,102 persons was examined and 12 (0.24%) were found to be microfilaria (mF) carriers and all of them were treated.

Screening of pre-school and school children:

Six "Anganwadi" centres in three wards of Shertallai South panchayat were covered to screen pre-school children (below 5 years). None of the 194 children examined was found to harbour mF of *B. malayi*. Of the 3,041 smears, collected from the school children covering 18 schools in nine panchayats 55 (1.81%) were found positive for mF.

Sample blood survey:

Parasitological survey was carried out through door to door visits in Mararikulam North panchayat where monthly and biannual single dose mass DEC therapy were carried out by the trained community volunteers. Out of 484 persons examined from monthly mass DEC area, 5 (1.03%) were found to be positive for mF and in the biannual area there were 15 (1.63%) microfilaraemic individuals out of 920 examined.

Health camp:

A health camp was conducted in Shertallai South panchayat by which 523 and 338 persons

benefited by getting themselves examined clinically and parasitologically respectively. As many as 73 (13.96%) persons had the clinical symptoms of filariasis and 11 (3.25%) had mF of *B. malayi.*

1.5.4. Filariasis Detection and Treatment Centres (FDTC):

During the reporting period 21 more FDT centres started functioning and 42 community volunteers were given training in blood smear collection and delivering selective DEC therapy. At present there are 96 such centres manned by trained volunteers of community organisations, FILCO as well as SFCC members. As many as 12,388 smears were collected from these centres during the year 1990 and were examined for mF at VCRC. Treatment was given to all the 111 mF carriers detected through these centres. Treatment follow-ups were also carried out by these volunteers to ensure the completion of selective therapy.

Through Health Care Delivery system, out of 4 lakh population living in the target area, 1,16,600 persons were examined so far for microfilaraemia and 33,048 for clinical manifestations of filariasis. As many as 1,710 mF carriers and 7,821 clinical cases of filariasis were detected and treated with selective therapy (Table 1.6).

1.5.5. Mass drug therapy:

Mass drug therapy continued to be one of the major activities of VCRC in liquidating the parasite load. Mass drug therapy was also initiated in Mararikulam North Panchayat, involving trained community volunteers.

Annual single dose (6mg/kg body wt) mass DEC therapy, targeted to cover a population of 28,411 in Shertallai South Panchayat was extended for the third round. House visits were made and 20,401 persons were administered with a single dose of DEC. All the fifteen schools in this area were covered and a total of 3,986 children was treated. Mass DEC was

Table 1.6.

Cumulative number of people screened and number treated for filariasis through different approaches.

Approach	Parasitological			Clinical		
	No.examined	No. +ve	mF rate (%)	No.examined	No. +ve	Disease rate (%)
Sample surveys	19,568	332	1.69	4,081	335	8.2
Mass surveys	4,346	218	5.02	5,420	565	10.42
Community Filariasis Detection Camps (192)	30,477	413	1.35	-	-	-
Health Camps (18)	7,453	135	1.80	9,635	664	6.90
School Filariasis Detection Camps (18)	3,041	55	1.81	-	-	-
Filariasis clinc at VCRC	20,868	265	1.27	13,912	6,257	44.98
Filariasis Detection & Treatment Centres	30,847	292	0.95	-	-	-
Total	1,16,600	1,710	1.47	33,048	7,821	23.66

Figure in parenthesis denotes number of camps conducted

also given to 187 pre-school children through 'Anganwadi' centres. The coverage in this third round was 86.49%. Side reactions following DEC administration were noticed in 199 (4.99%) school children, 1 (0.53%) pre-school children and 31 (0.15%) adults and the average incidence of side reaction was 0.94%.

As the children form the target group for liquidating parasite load, mass drug administration with single dose DEC was also carried out in schools to cover them. Out of 12,184 children in 14 schools, 10,622 (87.18%) were administered with mass DEC. Side reactions were noticed in 763 (7.18%) children and were treated with appropriate drugs.

Mass drug administration at monthly low dosage of 6mg/kg body wt was carried out by fifteen trained volunteers in two wards of Mararikulam North panchayat, covering a population of 600. Pretreatment mF prevalence in this area was 2.56% and it was brought down to 1.03% following mass therapy, with a percentage reduction of 59.77%. Similarly the vector infection was brought down from12.77% to 6.67% following the therapy. The vector infectivity rate prior to therapy was 2.13% and after therapy no infective vector mosquito was recorded.

The fourth round of biannual single dose DEC therapy was administered by the community volunteers in four wards of Mararikulam North panchayat with a population of 7,507. As many as 7,076 persons were treated, the coverage being 94.26%. Microfilaria prevalence was brought down from 3.87 to 1.63% following therapy with a percentage reduction of 57.88%. Vector infection and infectivity rates were 8.6 and 2.15% during the pre-control period and were brought down to 6.67 and 0 respectively in the post control period in this area.

1.5.6. Miscellaneous studies:

Community acceptance of DEC therapy:

Microfilaria carriers, detected through different case detection approaches were intimated and distributed with one full course of DEC. The completion of the course was checked by tablet counting method. Out of 69 cases followed, 51 (73.9%) completed the course regularly, 14 (20.2%) were found to be irregular but completed, 3 (4.35%) discontinued and 1 (1.45%) refused to accept the therapy. Development of fever was the main cause reported for the discontinuation of treatment. Thus this preliminary study indicates that DEC is acceptable to the majority of the people.

Natural history of elephantiasis:

Filarial patients with persistent oedema associated with skin changes (elephantiasis) were longitudinally followed to understand the natural history of this manifestation. The mean age of the patients with elephantoid changes was 55.9 years. The mean duration of oedema was 27.6 years and 19.87 years among elephantoid cases with warty outgrowth and nodules respectively. The onset of warty outgrowth was reported to be after an average of 22.4 years of oedema development and it was 12.62 years in the case of nodular outgrowth. Frequency of filarial fever among elephantoid cases with only skin changes was 11.44 per year, whereas in elephantoid cases with warty and nodular outgrowths it was 14.6 and 15.0 per year respectively. Studies have also been initiated to isolate the pathogens from elephantoid cases with secondary infections, culture them to identify their spectrum as well as sensitivity to different antibiotics. Attempts are also being made to evolve an appropriate therapy to control secondary infections among elephantiasis cases.

Variation of microfilarial counts in blood samples:

The number of microfilariae found in the multiple smears with same volume of blood taken from 18 mF carriers were analysed to find their variation. The number of mF ranged from 1 to 367 in different mF carriers. Statistical analyses showed that there was no significant difference between mean mF counts obtained from different samples from the same individual. The probability of getting the mF in the first smear was found to be 100% whereas it was 94% and 78% in the second and third smears respectively. Further observations are in progress.

Clinical follow-up studies among school children:

To understand the course of development of clinical manifestations and to find out the incidence of new clinical cases, a cohort of 1,812 children, aged between 6 and 16 years was followed longitudinally at monthly intervals. Initial clinical examination showed that 298 (16.45%) children had history of clinical symptoms of filariasis and two cases (0.11%) were

clinically positive for filarial lymphoedema. Second month follow-up observation showed that only 23.83 % of the 298 cases with history of filarial symptoms have reported to have filarial fever. No incidence of new clinical cases was observed within the period of 30 days. Further observations are in progress.

2. MALARIA STUDIES IN KORAPUT DISTRICT OF ORISSA STATE

In the previous years, malaria studies in Koraput district were directed mainly towards investigating the reasons for the persistence of malaria problem from the view point of (i) vector, (ii) parasite, (iii) human ecology and (iv) operational aspects. During the reporting year, studies on the distribution of Anopheline species in different parts of the district, bionomics of the vectors, i.e., man biting habit, vectorial capacity, etc. and surveys on *P. ovale* distribution were continued. Studies on the biological features of *P. falciparum* in natural infections were initiated. Field trials of alternate control strategies were also undertaken and the results are presented here.

2.1. EPIDEMIOLOGY

2.1.1. Distribution of *Plasmodium ovale*:

Survey of an additional 23 villages covering a population of 6,305 was done and none of the 1,501 blood smears examined was positive for *P. ovale*. However a survey conducted in June, 1990 at a dam construction site, to determine the rate of malaria infection in 1,046 persons, revealed that 9 persons were infected with *P. ovale*.

2.1.2. Distribution of malaria and sickle cell trait in Koraput district:

Genetic variations are known to play a role in determining the endemicity of malaria in the tribal areas. Therefore, it is important to find out whether, the prevalence of malaria among the tribals varies due to their genetic characteristics or due to environmental factors. Prevalence of sickle cell trait in various tribal as well as non-tribal population was monitored to investigate the possible association between the frequency of occurrence of the sickle cell trait and the intensity of malaria transmission in different geoclimatic areas of the district. A total of 18 villages, representing a population of 7,555 was surveyed in Jeypore zone. Among the 1,253 samples examined, 130 (12.5%) were positive for presence of sickled RBCs. In Malkangiri zone a population of 58,730 was surveyed and 710 samples were collected of which 55 (7.7%) showed sickling of RBCs. Since the sample size is too small, analysis to establish correlation could not be carried out and data collections are being continued.

2.1.3. Biology of *P. falciparum* gametocytaemia in natural infections:

Since gametocytes are the most important determinant of transmission potential of human host to vector population, understanding their biology is of utmost importance for rationalising control measures. Therefore, a study was conducted to determine (i) the time lag between appearance of asexual forms and that of gametocytes in peripheral blood, (ii) the duration of persistence of gametocytaemia, (iii) the gametocyte conversion rate, (iv) the peak gametocyte count and (v) sex ratio.

Twenty two persons were followed up regularly on every alternate days for 30 days and thereafter at weekly intervals to study the appearance and disappearance of gametocytes in the peripheral circulation. The minimum average time taken for the appearance of gametocytes was found to be 9.3 days in children (below 15 years) and 8.9 days in adult (above 15 years) after the detection of asexual parasitaemia. The difference between two groups is not significant ($T = 0.11$; $P = 0.92$). This observation has an important bearing in deciding the timing of administration of gametocytocidal drug like primaquine for *P. falciparum* cases. Since primaquine is excreted rapidly (short half life of 6-8 h) the usage

of this drug needs to be rationalised. Since chloroquine in proper dosage will clinically cure and eliminate asexual parasites in most of the cases, a drug like primaquine which is scarcely available and toxic, specially to G-6-PD enzyme deficient persons, its usage should be restricted to slide proven gametocyte carriers only.

Duration of gametocytaemia in peripheral circulation was found to be ranging from 1 to 32 days. The gametocyte conversion rate on an average was 0.74% and 2.04% in children and adults respectively. The mean sex ratio of micro : macro gametocytes was 1:2.6 and 1:6.2 in children and adults respectively. Two cases (3 yrs. old male and 6 yrs. old female) out of the 22 followed did not produce any gametocyte at all during the entire followup period of 155 days and 134 days respectively. The reason for this phenomenon is not clear. This could suggest the presence of a different parasite strain in this area.

Among the four human plasmodia, only the gametocytes of *P. falciparum* sequester within 24 h of invasion of red blood cell. They mature in the sinusoids of spleen and blood marrow and are released to peripheral blood circulation as mature stage V gametocytes. This phenomenon takes about 10 days after patent parasitaemia under natural conditions. However in a hyperendemic village of Malkangiri, Koraput district, three cases with immature stages of *P. falciparum* gametocytes (Stages I, III & IV) along with stage V mature gametocytes were detected in natural infection. All these cases were children under 4 yrs. of age.

2.2. VECTOR BIOLOGY AND ECOLOGY

2.2.1. Mosquito fauna:

Survey of mosquito fauna in different physiographic regions of Koraput district was continued. Twenty three anopheline species were recorded earlier (Annual Report 1989). During this year, three more species, *An. aitkeni, An. moghulensis* and *An. peditaeniatus* were recorded. A new variety in *An. annularis* was recorded and named as *odissi* (VCRC). This variety has an extra black band in both palpi and hind tarsi.

In addition, 48 species and 2 varieties belonging to 8 genera of tribe Culicine and one species belonging to tribe Megarhinini have been recorded (Table 2.1).

Table 2.1.

Mosquitoes of Koraput district collected from different breeding habitats (other than Anophelines).

Sl.No.	Name of the species
1.	*Aedes (Aedimorphus) jamesi*
2.	*Aedes (Aedimorphus) pipersalatus*
3.	*Aedes (Aedimorphus) stenoetrus*
4.	*Aedes (Christophersiomyia) thomsoni*
5.	*Aedes (Finlaya) albolateralis*
6.	*Aedes (Finlaya) aureostriatus* var *kanaranus*
7.	*Aedes (Finlaya) feegradei*
8.	*Aedes (Finlaya) gubernatoris*
9.	*Aedes (Mucidus) scatophagoides*
10.	*Aedes (Neomelaniconion) lineatopennis*
11.	*Aedes (Stegomyia) albopictus*
12.	*Aedes (Stegomyia) craggi*
13.	*Aedes (Stegomyia) vittatus*
14.	*Armigeres (Armigeres) kuchingensis*
15.	*Armigeres (Armigeres) subalbatus*
16.	*Coquillettidia (Coquillettidia) crassipes*
17.	*Culex (Culiciomyia) nigropunctatus*
18.	*Culex (Culex) barraudi*
19.	*Culex (Culex) bitaeniorhynchus*
20.	*Culex (Culex) epidesmus*
21.	*Culex (Culex) fuscocephalus*
22.	*Culex (Culex) gelidus*
23.	*Culex (Culex) mimeticus*
24.	*Culex (Culex) mimulus*
25.	*Culex (Culex) nilgiricus*
26.	*Culex (Culex) quinquefasciatus*
27.	*Culex (Culex) sitiens*
28.	*Culex (Culex) tritaeniorhynchus*
29.	*Culex (Culex) vishnui*
30.	*Culex (Culex) whitmorei*
31.	*Culex (Eumelanomyia) castrensis*
32.	*Culex (Eumelanomyia) khazani*
33.	*Culex (Eumelanomyia) malayi*
34.	*Culex (Eumelanomyia) pluvialis*
35.	*Culex (Lophoceraomyia) minutissimus*
36.	*Culex (Lophoceraomyia) uniformis*
37.	*Culex (Lutzia) fuscanus*
38.	*Culex (Lutzia) raptor*
39.	*Culex (Lutzia) vorax*
40.	*Heizmannia (Mattinglyia) discrepans*
41.	*Mansonia (Mansonioides) annulifera*
42.	*Mansonia (Mansonioides) uniformis*
43.	*Orthopodomyia andamanensis*
44.	*Orthopodomyia anopheloides* var *maculata*

45. *Toxorhynchites (Toxorhynchites) splendens*
46. *Uranotaenia (Pseudoficalbia) bicolor*
47. *Uranotaenia (Pseudoficalbia) luteola*
48. *Uranotaenia (Pseudoficalbia) recondita*
49. *Uranotaenia (Uranotaenia) annandalei*
50. *Uranotaenia (Uranotaenia) orientalis*

2.2.2. Biting rhythm and nocturnal activity of malaria vectors:

In the earlier report (1989), the species composition of man biting anopheline population and man biting rate (number biting per man per night) of *An. fluviatilis, An. culicifacies* and *An. annularis* in differ ent seasons were given for Jeypore zone. While these studies were continued in Jeypore, they were also extended to Malkangiri, another geoclimatic zone of Koraput district. The indoor biting rate of *An. fluviatilis, An. annularis* and *An. culicifacies* in the two zones are given in Table 2.2.

Table 2.2.

Man biting rate of *An. fluviatilis*(I), *An. annularis*(II) and *An. culicifacies* (III)

	Jeypore			Malkangiri		
Month	I	II	III	I	II	III
Dec 1988	4.5	11.5	0.0	53	0	0
Jan 1989	1.7	2.5	0.0	20	0	0
Feb	1.5	0.0	0.0	2	0	0
Mar	2.7	0.5	0.3	3	0	26
Apr	16.0	0.5	0.0	7	0	0
May	2.5	0.0	0.5	10	0	3
Jun	5.5	0.5	0.5	1	0	0
Jul	7.0	0.0	0.0	9	0	0
Aug	3.0	0.5	0.5	11	0	3
Sep	11.5	1.0	0.0	3	0	1
Oct	8.0	2.0	0.0	27	0	2
Nov	9.0	4.5	0.0	28	0	0
Dec	7.5	1.0	0.0	7	0	0
Jan 1990	2.5	N D	0.0	22	0	0
Feb	4.0.	N D	0.0	3	0	0
Mar	3.0	N D	0.0	13	0	0
Apr	1.0	N D	0.0	4	0	1
May	2.5	N D	0.0	2	0	0

ND - Not done.

In both the zones *An. fluviatilis* was biting indoors in all the months. However, the season of the peak biting densities was different in the two zones. While in Jeypore, peak density was observed during rainy season, the same was found in winter in Malkangiri which coincides with peak malaria incidence. The man biting rate was higher in Malkangiri zone as compared to Jeypore.

In both the zones, outdoor biting of *An. fluviatilis* was observed in all seasons and the biting rate ranged from 0.5 to 3.0. During summer about 10-15% of the inhabitants sleep outdoors throughout the night and biting of *An. fluviatilis* was observed throughout the night. The outdoor biting rate did not differ markedly from that of indoors. During winter, majority of the people sleep indoors, after spending the early part of the night outdoors near the fire (Plate IV, A). A few, however, sleep outdoors keeping the fire burning throughout the night. The outdoor biting activity of *An. fluviatilis* coincided with the sleeping habits of the villagers and was restricted to early hours of the night when most of the people stayed outdoors. During the rainy months, nobody sleeps outdoors but *An. fluviatilis* biting occurred when human hosts were made available.

The biting activity of *An. annularis* was studied only in Jeypore zone where this species was recorded throughout the year. The biting rate was highest in the month of December. No biting activity was observed in the months of February, May and July. Biting rates were low during the rest of the year and rose gradually in October and November. *An. culicifacies* biting was observed mainly in the villages in Malkangiri area. The biting rate was highest in the summer month of March (26 per man per night) and to a limited extent during other months of summer and rainy seasons.

Data on biting cycle show that *An. fluviatilis* biting occurred throughout the night in all seasons and its biting activity was more in the II and III quarters of the night i.e., from 2100 hrs to 0300 hrs. However, in Jeypore zone in winter, highest activity was observed in the I quarter and the activity was much reduced in the III and IV quarters. The shift in the biting activity to the I quarter in winter season was probably due to the very low temperature in the latter parts of the night (Table 2.3).

Table 2.3.

Number of *An. fluviatilis* obtained from all night man biting collections in different quarters of the night. (18.00-06.00 Hrs.).

Place	Season	Quarter	No. of female collected	Temperature (°C) Min.	Max.
Jeypore	Summer	I	2	27.8	29.7
		II	27	26.3	27.8
		III	31	24.4	25.7
		IV	7	22.5	23.8
	Rainy	I	2	23.6	24.6
		II	26	23.0	23.6
		III	24	22.2	22.8
		IV	7	21.5	21.9
	Winter	I	37	16.3	19.3
		II	19	14.4	15.6
		III	4	12.8	13.7
		IV	3	11.9	12.5
Malkan-giri	Summer	I	5	26.6	29.8
		II	11	24.1	27.6
		III	24	23.1	24.9
		IV	2	22.3	23.8
	Rainy	I	1	24.9	26.8
		II	22	24.3	26.1
		III	25	23.8	24.9
		IV	2	23.4	24.3
	Winter	I	17	18.8	25.3
		II	70	16.6	17.7
		III	59	15.7	17.9
		IV	11	14.8	16.5

The biting activity of *An. annularis* was also found in first quarter of the night in winter. The data for summer and rainy season could not be analysed due to small numbers collected. The biting activity of *An. culicifacies* in summer starts at dusk hour and is continued upto 0300 hrs, after which no activity was observed. In other seasons the number collected was too small (Table 2.4).

Table 2.4.

Number of *An. culicifacies* and *An. annularis* collected from indoor man biting collections in different quarters of the night (18.00-06.00 Hrs.).

Season	*An. culicifacies*				
	I	II	III	IV	Total
Summer	9	12	9	0	30
Rainy	1	5	0	0	6
Winter	0	0	0	0	0
	An. annularis				
Summer	1	1	0	1	3
Rainy	2	2	1	2	7
Winter	28	2	2	4	36

Majority of *An. fluviatilis* collected from human baits were either unfed or fully fed. Only 2.9% in Jeypore area and 0.9% in Malkangiri were found to be semi-gravid indicating that they needed refeeding for the completion of gonotrophic cycle. This phenomenon in *An. fluviatilis* either in the same night or on the successive nights has been reported earlier. The proportion of *An. annularis* landing on man in semi-gravid condition was higher (15.2%), which confirms the refeeding of this species within the same gonotrophic cycle. Though some semi-gravid specimens of *An. culicifacies* were also collected, the refeeding phenomenon could not be confirmed.

The number of *An. fluviatilis, An. culicifacies* and *An. annularis* collected from night resting collections in different quarters of the night in different seasons in both Jeypore and Malkangiri areas was found to follow the same pattern as found in all night man landing collections. Among *An. fluviatilis* collected 72.7% and 50.8% were unfed and 26.8% and 21.3% were fully fed respectively in Jeypore and Malkangiri zones. The predominance of unfed ones in night rest-

ing collection in exophilic mosquitoes indicate that these mosquitoes entered the houses from their day time shelters and rest on the wall for some time before feeding. A proportion of fully fed collected also shows that not all mosquitoes leave the hut immediately after feeding and some do rest after feeding also. In contrast, 71.3% of the total *An. annularis* collected were fully fed, 10.3% unfed, 13.78% semigravid and 4.6% were gravid which is expected in endophilic mosquitoes.

The study shows that *An. fluviatilis* is the major man biting mosquito in villages of both Jeypore and Malkangiri areas. In some villages *An. annularis* and *An. culicifacies* also bite man but the biting rate was very low due to their predominant zoophilic habit. Since *An. fluviatilis* rests inside the human dwellings before and after feeding on man and thus there is still possibility that this species is exposed to residual sprays.

2.2.3. Vectorial capacity:

Vectorial capacity is an important index of transmission potential which could be used in stratifying areas for planning control strategies. Therefore, the vectorial capacity of *An. fluviatilis, An. culicifacies* and *An. annularis* was estimated for the three plasmodial species viz., *P. falciparum, P. vivax* and *P. malariae* in different seasons. The man biting rate, probability of survival through one day and estimated vectorial capacity of *An. fluviatilis* and number of malaria cases for the three seasons are summarized in Table 2.5.

There is wide variation in the vectorial capacity of *An. fluviatilis* between the two zones. A higher value has been obtained for Malkangiri zone which is due to higher man biting rate and human blood index. The seasonwise analysis for Malkangiri and Jeypore zones shows that vectorial capacity was higher during rainy season. It was followed by winter season in former while by summer in the latter. In Malkangiri zone, man biting rate was much higher in cooler months than rainy season, but this did not result in the corresponding increase in vectorial capacity since the sporogonic period was longer in cooler temperatures. In both Jeypore and Malkangiri zones a good correlation ($R = 0.9594$; $P = 0.002$) between the estimated vectorial capacity values and number of malaria cases particularly *P. falciparum* was observed.

The vectorial capacity of *An. annularis* was calculated only for cold and rainy seasons. The estimated value for *P. falciparum* was relatively higher in cold season (0.002) during which, the survival rate of the species was also more as compared to rainy season. In this hilly tract, this species is found in good numbers only in villages of Jeypore zone where ponds are present.

Table 2.5.

The average man biting rate (ma), probability of survival through one day (p) and estimated values of vectorial capacity of *An. fluviatilis* along with the number of malaria cases in different seasons.

Seasons	Zones	'ma'	'p'	Vectorial capacity			No.of malaria cases		
				Pf.	*Pv.*	*Pm.*	*Pf.*	*Pv.*	*Pm.*
Summer	Jeypore	3.71	0.76	0.147	0.256	0.086	37	7	4
(Mar - June)	Malkangiri	5.25	0.79	1.421	1.795	0.889	29	7	0
Rainy	Jeypore	7.38	0.82	0.361	0.537	0.198	60	8	0
(Jul - Oct)	Malkangiri	12.50	0.85	3.025	4.197	1.854	131	24	0
Winter	Jeypore	3.35	0.82	0.049	0.110	0.019	35	5	2
(Nov - Feb)	Malkangiri	19.63	0.86	2.379	4.362	1.118	108	15	1

HBI: 0.26 for Jeypore & 0.84 for Malkangiri; Gonotrophic cycle: Summer - 2 days, Rainy & Winter - 3 days.
Pf : P.falciparum; Pv : P.vivax; Pm : P.malariae

The man biting rate is generally very low and only one sporozoite positive was found in a foot-hill village earlier. Therefore, it is concluded that *An. annularis* is only a localised vector playing role in malaria transmission when the density is very high.

An. culicifacies biting occurred only during rainy and summer seasons and the respective vectorial capacity values to these seasons for *P. falciparum* were 0.117 and 0.017. *An. culicifacies,* though incriminated as vector in other parts of Orissa, in this area its vectorial capacity is low even when the density was high. This was due to its poor man biting habit. In cold season the density reduces to a very low level and in higher altitudes prevalence is very low in most of the months. Therefore, it could be concluded that this species plays a secondary role in transmission.

The difference in the vectorial capacity calculated for different plasmodial species is a reflection of the difference in the estimated values of sporogonic period. As a result, higher values have been obtained for *P. vivax,* which has a shorter sporogonic period than *P. falciparum*. This, however, has a striking difference when the parasite species prevalence in the villages is considered. *P. falciparum* has been the dominant species in the two zones, forming 70-80% of the total malaria cases. The comparatively longer duration of gametocytaemia in untreated persons and lesser stimulation of immune response have been reported to favour the preponderance of *P. falciparum* in areas where there is prolonged transmission. The vectors may also have different susceptibility to the different plasmodial species, which will be contrary to the assumption made in the estimation of vectorial capacity.

2.2.4. Dispersal of Anopheline vectors:

In a preliminary study in terraced rice fields, immatures of *An. fluviatilis, An. culicifacies, An. annularis, An. jeyporiensis* and *An. maculatus* were obtained in significant numbers upto one kilometer distance from human habitation (Annual Report, 1989). This was higher than what was reported earlier. Based on this information, a further study has been undertaken in the same area since June 1989.

Rice plots were selected at different distances, 50, 500, 1000 and 1500 m from the village and larval breeding were monitored. Anopheline larvae were collected and reared to adults and identified. The results showed that the density of *An. fluviatilis* immatures was higher upto one kilometer distance, after which it decreased.

An. fluviatilis exhibits a higher degree of exophily and the pit shelters are found to be the most preferred outdoor resting shelters of this species (Plate IV, B). Therefore artificial pit shelters were dug at the well shaded earthern banks along the sides of streams at different distances from the village in concentric circles in all directions. Resting adults were collected from these shelters at fortnightly or weekly intervals. The number of adults collected from each shelter and their gonotrophic stages and blood meal sources were recorded. So far a total of 134 *An. fluviatilis* with all gonotrophic conditions was collected, of which 90% were obtained within 500 m (in all directions). The maximum distance *An. fluviatilis* dispersed was 1000 m beyond which adults were not found. Precipitin results show that 23.5% of the samples were positive for human blood and 76.5% for bovine blood.

2.2.5. Susceptibility status of malaria vectors to DDT, BHC and malathion:

Malkangiri sub-division received indoor residual spray with DDT from 1958 to 1971 and with BHC from 1972 onwards. Since insecticide pressure was different from Jeypore zone, susceptibility status of Anopheline vectors was monitored. *An. culicifacies* was found resistant to DDT, BHC and malathion. *An. fluviatilis* was resistant to BHC but susceptible to DDT. This is the first report of resistance in this species to BHC. Resistance to BHC can be attributed to selection pressure of the above insecticide used for a long period (Table 2.6).

2.2.6. Vector competence of *An. fluviatilis, An. culicifacies* and *An. annularis* to human plasmodia:

The efficiency of different vectors in transmitting different plasmodia determines the dynamics of malaria transmission. Vector susceptibility to a particular parasite species is an inherent characteristic of the species. Hence its susceptibility to plasmodia needs evaluation to elucidate its role in malaria transmission. Gametocyte carriers were selected by doing a mass blood survey. The absolute number of asexual and sexual parasites were counted against 200 RBC. The sex ratio was calculated. The laboratory colo-

Table.2.6

Percentage of mortality in vector species exposed to diagnostic dosages of HCH, DDT and malathion.

Species	Insecticide	Exposure Period	No.exposed	Corrected mortality (%)
An. fluviatilis	BHC(0.4%)	1 hr.	90	66.7
		2 hr.	85	71.8
		4 hr.	100	91.0
		6 hr.	100	98.2
	DDT(4.0%)	1 hr.	60	96.7
		2 hr.	30	100.0
	Malathion (5%)	1 hr.	45	95.6
An. culicifacies	BHC(0.4%)	1 hr.	30	6.6
		2 hr.	30	3.3
		4 hr.	30	10.0
		6 hr.	30	20.0
	DDT(4.0%)	1 hr.	30	0
		2 hr.	30	3.3
		4 hr.	30	0
		6 hr.	30	20.0
	Malathion (5%)	1 hr.	30	63.0
		2 hr.	30	70.0
		4 hr.	45	84.0
		6 hr.	30	100.0

nized 2 days old unfed mosquitoes were allowed to feed on volunteers. Three volunteers (gametocyte count ranging from 160 to 7,040 per cu.mm.) were exposed to the bite of *An. fluviatilis* and one (gametocyte count: 160 per cu.mm.) to *An. culicifacies*. Out of 90 *An. fluviatilis* released, 20 fed on the volunteers of which three were found positive for sporozoites and oocysts when dissected on eleventh day after feeding and one was positive for oocyst on eighth day. Out of 40 *An. culicifacies* released, nine fed and one was positive for oocyst on eleventh day.

2.3. FIELD TRIALS:

2.3.1. Evaluation of withdrawal of DDT residual spraying in a low receptive area:

Residual spraying of DDT carried out by NMEP was not effective in reducing malaria transmission because of several known reasons. Resource constraint is considered to be the major obstacle in malaria control. Hence, optimising the operation within the available resources is necessary by selectively applying this control measure in more problematic and

responsive areas and withdrawing the same from low receptive areas. Therefore, a study was under taken in Hardoli section of Borigumma PHC in Koraput district to evaluate the epidemiological change after withdrawing the DDT residual spray.

There are 17 villages in Hardoli section with a population of 6,300 situated in plain area. The area has been divided into two comparable groups, one as the experimental area with 10 villages having 3,570 population and other as control area with 7 villages having a population of 2,734.

Mass blood survey carried out in January-February 1990 showed that the malaria prevalence in control and experimental villages was comparable, and the slide positivity rate was 11.5 and 9.6 respectively.

Fortnightly fever surveillance was initiated from March 1990. The monthly incidence of indigenous malaria cases ranged from 0.73 to 2.19 per thousand in control and 0.56 to 4.48 per thousand in experimental villages. Relatively higher incidence was observed in the months of June and July in adult age group, and most of the cases were imported from Muran Dam site.

The 1st round of spray was done by NMEP on 17th and 18th August 1990 in control group of villages and the experiment group was left unsprayed. A survey was done on the next day of spray to assess the coverage. Only 15% of the rooms were completely sprayed, 2% received partial spray and 83% were left unsprayed. All the sprayed rooms were mud-plastered within 15 days.

The incidence has been slightly higher in the experimental group compared to the control. There was a decline in the incidence in the sprayed villages in September. In October no *falciparum* case was recorded. Since only a small number of cases occurred throughout the study period and the spray operation was carried out when the incidence was showing a declining trend in all villages, it is difficult to comment upon the effect of spray conclusively.

Density of *An. fluviatilis*, the major vector was very low in both control and experimental villages. Indoor resting density of *An. culicifacies* in control and experimental villages before and after spraying is given in Fig:2.1. The density was on a declining trend from August onwards in both control and experimental

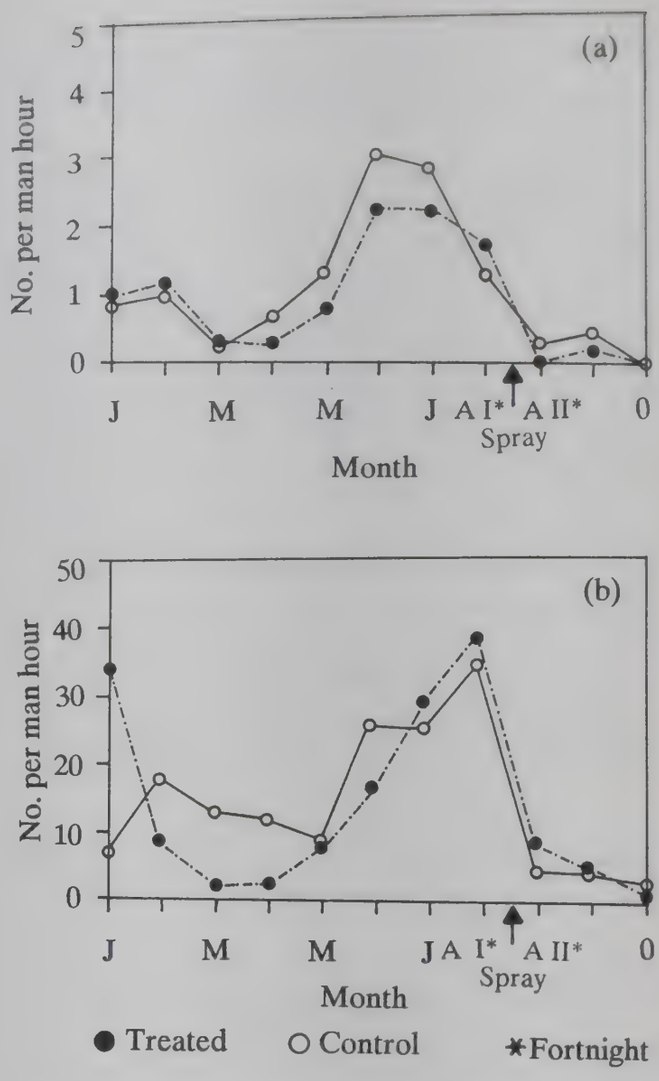


Fig. 2.1 Density of *An. culicifacies* in experimental and control villages in human dwellings (a) **and cattle sheds** (b).

villages due to the seasonal influence. Since spray was done when the density was already on a declining trend, the effect of spray could not be assessed properly. There was no change in resting of *An. culicifacies* from indoors to outdoors or from cattle-sheds to human dwellings. The susceptibility tests were conducted 2 days and 3 weeks after spray in control and experimental villages. *An. culicifacies* was resistant to DDT and no marked difference was noticed between the two groups of villages.

2.3.2. Impact of indoor DDT residual spraying on density, longevity and behaviour of malaria vectors where mud-plastering is deferred:

Eventhough *An. fluviatilis* is mainly exophilic, a considerable proportion of the population rests indoors for partial completion of the gonotrophic cycle. It was

also observed that *An. fluviatilis* rests on walls before and after feeding. *An. fluviatilis* is susceptible to DDT and BHC and the behaviour pattern indicates that this species does not avoid sprayed surfaces. Inspite of these factors the indoor residual spray has failed to interrupt the transmission mainly due to improper spray and mud plastering. Therefore a study was undertaken to evaluate the efficacy of proper DDT spray. Two identical, malarious tribal villages viz., Maliguda and Kunjei of Tankua section under Rabanaguda PHC have been selected for this study. Both are foot-hill villages and are situated at the same altitude. Breeding habitats were similar in these two villages. Preliminary collections showed that the vector density did not vary significantly. Both parasitological and entomological evaluations were carried out in these two villages.

First round of spray was done by VCRC in the month of August 1990 when the NMEP carried out the routine spray in the PHC area. The sprayed walls could be retained without mud-plastering only upto 22 days.

There was a marked reduction in the density of *An. culicifacies* in sprayed village upto two and half months as compared to the control village. The density of *An. fluviatilis* was too low to comment. Incidence of malaria showed a declining trend in sprayed village whereas in control village there was an increase.

2.3.3. Village scale trial with Lambdacyhalothrin impregnated bed-nets:

Malaria control by conventional residual insecticide spray has not produced desired result in the tribal belt of Koraput district. Living condition and customs of tribals such as, half open house, frequent mud plastering of walls, refusal to accept spraying are some of the reasons for ineffectiveness of spraying. In the past, trials with insecticide impregnated bednets have demonstrated reduction in density of malaria vectors. Therefore, a village scale trial was undertaken in tribal villages of Malkangiri area, from September 1989 to August 1990 to assess the impact of insecticide treated bed-nets on malaria vectors and transmission, in comparison with protection afforded by untreated bedbets.

These tribals are not in the habit of using bed-nets and they were motivated to do so. Nylon nets which can withstand wear and tear and have the advantage of retaining higher quantity of insecticides and for a longer duration were used. The nets were impregnated with lambdacyhalothrin 2.5% EC at the dosage of 0.025 g/sq.mt. The nets were distributed to villagers according to size of the family. Re-impregnation was carried out after six months.

Six months after the distribution of bed-nets the parasite rate in the treated village was reduced from 24.7 to 11.9%. (P.05) and further reduced to 7.2% after one year. The spleen rate decreased significantly in treated village after six months, while these parameters were unchanged in untreated village and increased in control village. The monthly slide positivity rate (SPR) in treated village remained at a low level throughout the study period except in the month of August when compared with village where untreated nets were supplied and in the village without nets (Fig.2.2). Infant positivity rate was analysed in the three villages for the assessment of interruption of transmission of malaria. In treated village it remained zero except in the month of January and July. A child born during study period was found positive for *P. falciparum* in the month of July, indicating fresh infection.

The per hut density of *An. fluviatilis* was reduced to zero immediately after introduction of impregnated nets and remained so throughout the study period. In the untreated and control villages it increased. The density of *An. culicifacies* also remained low in treated village as compared to the other two villages. The engorgement rate of *An. fluviatilis* was relatively high in untreated village which shows that there was no impact of untreated net on man biting habit. The man biting rate (MBR) of *An. fluviatilis* in the treated village was found reduced, whereas in control and untreated village, it shows an increasing trend (Fig.2.3). Total anophelines collected by pyrethrum space spray collection was low in treated village compared to untreated and control villages (Fig.2.4).

The bioassay result of unwashed and washed nets shows 100% corrected mortality during the entire study period. However, immediate mortality with 3 minutes exposure period was reduced after washing the nets. The reduction in the range of mortality may be due to the loss of potency of the insecticide during the washing. Nets retained the efficacy upto 24 weeks even after washing and drying in sun.

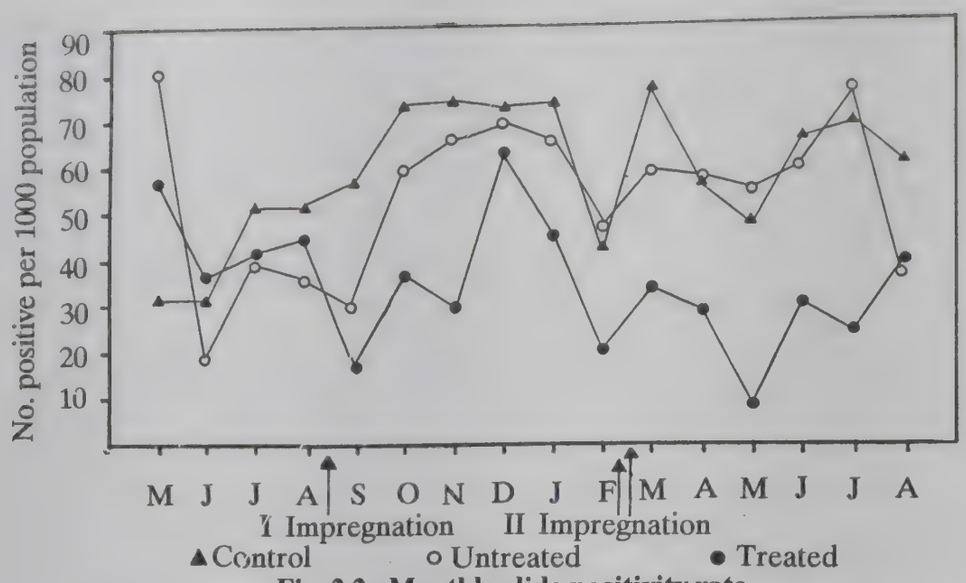


Fig. 2.2. Monthly slide positivity rate.

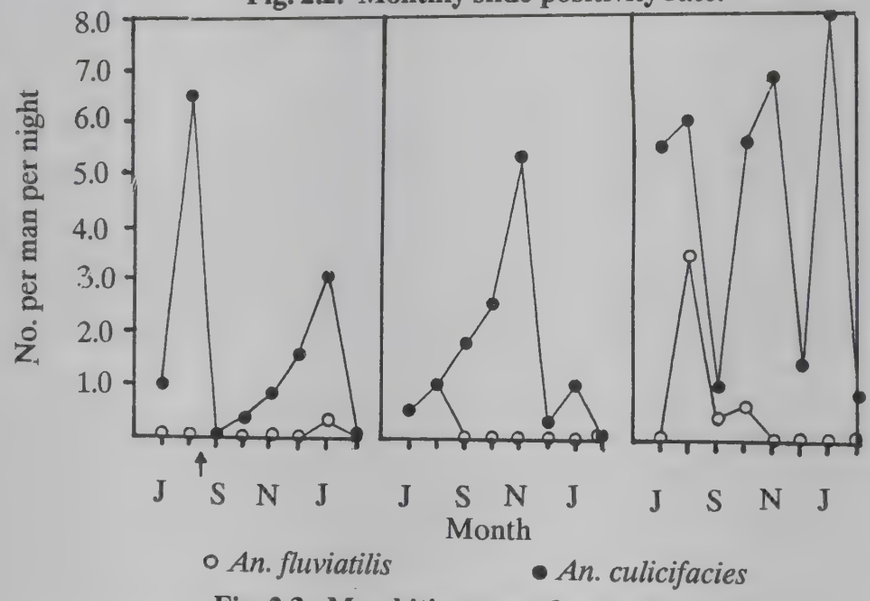


Fig. 2.3 Man biting rate of vector species.

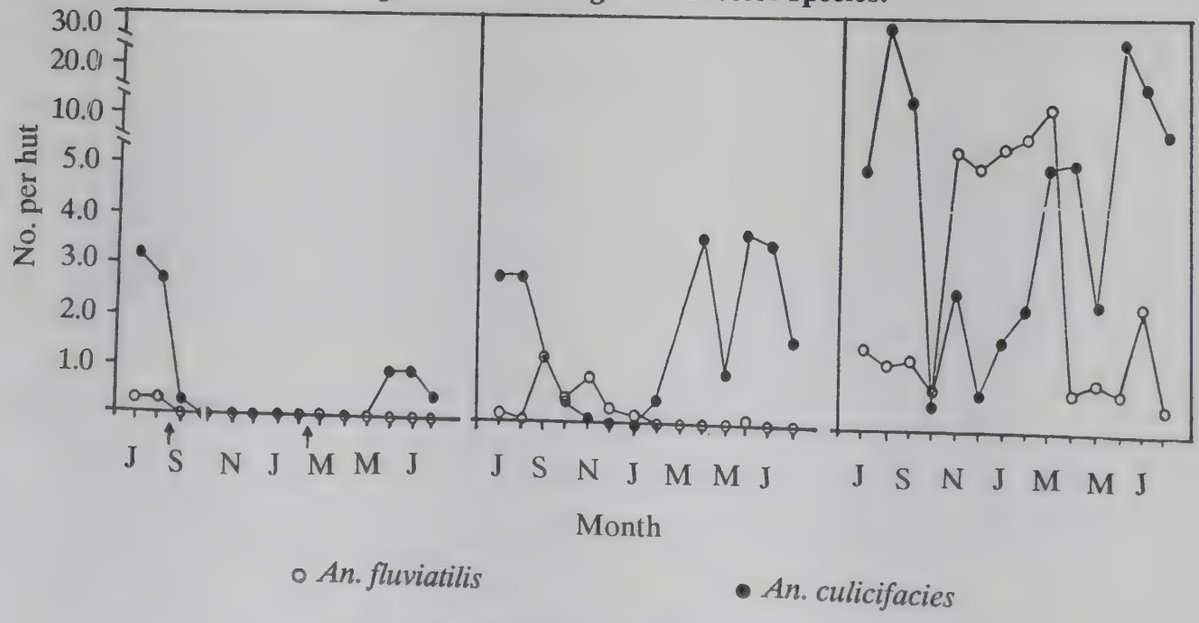


Fig. 2.4 Per hut density of vector species.

The tribals are poor and scantily clothed. Most of them cannot afford warm clothing and therefore sleep near fire during the winter. During hot summer months the weather is dry and therefore the nuisance of biting mosquitoes is reduced. Since the impact of impregnated bed-nets would depend upon the sleeping habit of the people, the same was monitored. In treated village 79% of population used the bed-net whereas in untreated village its use was 38%. The use of net in winter, summer and rainy season was 59, 86 and 92% respectively in treated village and its use was 5, 43 and 67% respectively in untreated village. In treated village as people realised the benefit and got habituated, some of them expressed a desire to purchase the nets.

3. STUDIES ON BANCROFTIAN FILARIASIS

3.1. PARASITE EPIDEMIOLOGY

3.1.1. Frequency distribution of parasite in the vector:

A number of theoretical and empirical studies have underlined the importance of quantifying the distribution of macroparasite abundance in the host population. Such distributions are characteristically *overdispersed* , and can often be described empirically by the *negative binomial* distribution. They reflect the dynamics of the host-parasite interaction and, in particular, can be used to provide evidence of density dependent constraints on parasite abundance. Specifically, an observed reduction in the degree of overdispersion (inversely measured by the *negative binomial* parameter *k*) with host age provides evidence of the operation of density dependent factors, which act to decrease the proportion of hosts with large parasite burdens. The two processes which can act in this way are parasite-induced host mortality and density dependent reduction in parasite survival within the host. A combination of statistical and analytical methods can then be used to explore the implications of these patterns.

The study examines the relationship between human and mosquito mf frequency distributions and the impact of vector as well as parasite stage on the observed frequency distribution of infection.

Entomological data comprise the results of a year round fortnightly resting collections, which yielded a total of 16,802 female *Culex quinquefasciatus* for parasitological dissection and age-grading, according to the number of previous egg layings (parity). There were 4332 nulliparous, 7930 one parous (1D), 3743 two parous (2D), 708 three parous (3D), 80 four parous (4D) and 9 five parous (5D) mosquitoes and of these 2481 were infected with filarial larvae.

Assuming that the vectors bite randomly with respect to host infection status, the frequency distribution of mf in freshly-fed nulliparous mosquitoes (i.e. after their first feed) should follow a mixed Poisson distribution, with an augmented zero class. The rationale for this model is as follows. If the mean density of mf in the peripheral blood of infected individuals follows a continuous probability density, h(m), then 'sampling' of this distribution by biting vectors (essentially a *Poisson process*) will lead to a Poisson mixture such as the *negative binomial* or the *Sichel* distribution for the positive counts, with a proportion of zeros which are due purely to the sampling process (i.e., negative, although the individuals bitten are mf positive). Since an often large proportion of humans will also be 'true' negatives (uninfected, with single sex infections, or immune), nulliparous mosquitoes which bite them will also be negative. This then leads to a Poisson mixture with excess zeros, which can be fitted to observed mf distributions by a zero-truncated fit, which is restricted to the positive counts. Based on this distribution, it is possible to test the null hypothesis that the mf distribution in fully fed nulliparous mosquitoes is the same as that in the human population and, in particular, shows a proportion of zeros due to biting of true mf negatives.

The expected distributions of later stages of parasite in older hosts is likely to be much more complex, since

they reflect a balance between development and death processes. Because of relatively small sample size of some of these stages, we compare the degree of overdispersion between stages using the variance to mean ratio, and test for differences between distributions using a linear model analysis of the prevalence of infection, and chi squared tests to compare the observed positive count frequencies with the expected ones.

(a) *Vector population structure*:

The instantaneous mortality rate of different parity stages of mosquitoes, calculated from the basic stage structure of the population are depicted in Fig. 3.1.

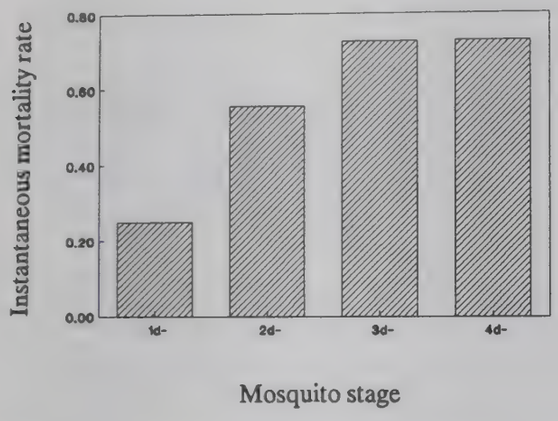


Fig. 3.1 **Estimates of the instantaneous mortality rate (per day) for different parity stages of *Cx. quinquefasciatus*. If the observed numbers in stages i and i+1 are n_i and n_{i+i}, then the mortality rate in stage i is estimated from $-1n(n_{i+i}/ni)/t_i$, where t_i is the duration of stage i.**

It illustrates an increase in mortality from 1D to 3D mosquitoes, which is typically observed in filariasis vectors. The higher mortality rate in later stages is likely to be, at least partly, due to parasite-induced host mortality.

(b) *Microfilarial distributions in human and vector populations*:

The observed relative frequency of mf positives in the human and vector population (nulliparous fully fed) are displayed in Fig. 3.2a, which also illustrates the striking similarity between the two distributions, and a slight tendency to underestimate single mf burdens in mosquitoes. The two distributions are significantly different at the 5% level ($\chi^2 = 21.9$; df = 19; p = 0.039), however this is essentially due to the under-representation of 1 mf class in the vectors. The overall microfilarial prevalence was 8.35% in the human population and 5.62% in nulliparous vectors. Again, these results are fairly similar, particularly given the likely underestimation of low mf prevalence in the vector. However, because of the huge sample sizes involved, the difference is statistically significant (humans, n = 25,867; vectors, n = 1904; z = 4.21; p = 0.001).

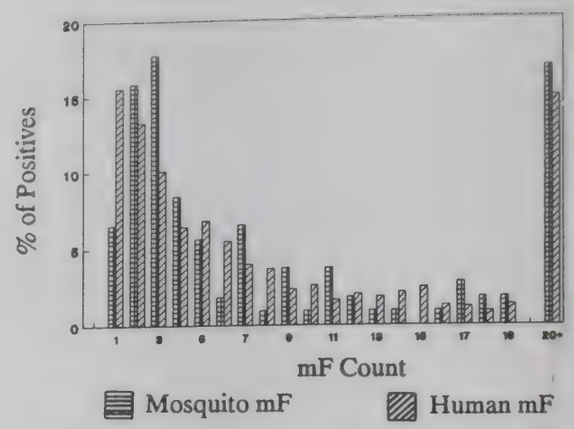


Fig. 3.2a **Frequency distribution of mF in humans and nulliparous fully fed mosquitoes.**

The observed positive mf distribution in nulliparous vectors with the expected counts from zero-truncated and non-truncated fits of the *Sichel* distribution to the human mf intensity data are depicted in Fig. 3.2b.

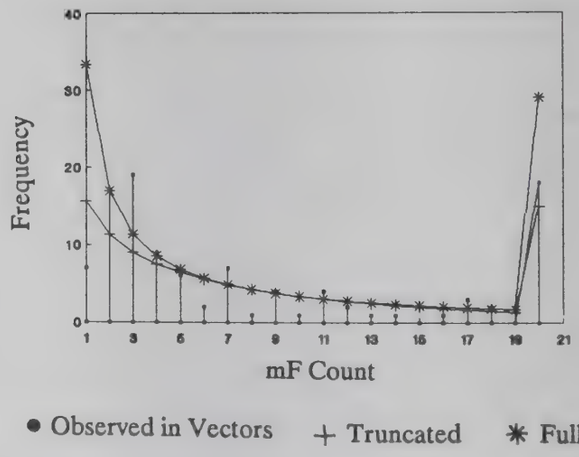


Fig. 3.2b **Comparison of observed mF distribition in fully fed mosquito with expected mF distribution in humans.**

The zero-truncated fit shows a similar fit to the observed counts in the vector as does the equivalent observed distribution (Fig. 3.2a). However, the non-truncated fit to the human data does not accord

with the observed distribution for the vector. In particular, the non-truncated fit is far more overdispersed than the observations, with a much longer tail of high counts. This lack of fit of the non-truncated distribution is also found for the human mf distribution and corroborates the hypothesis outlined above, that a proportion of mosquitoes are uninfected due to bites on true mf negatives in the human population.

(c) *Patterns of prevalence, intensity and overdispersion:*

Table 3.1 gives the variance/mean ratio of infection intensity in mosquitoes of different parity.

Table 3.1

Variance/Mean ratio of parasite density for different mosquito and parasite stages.

Larval stage	NP	1D	2D	3D
MF	27.33	28.81	56.92	20.07
L1	-	18.93	15.60	25.40
L2	-	18.29	13.17	9.41
L3	-	-	7.28	7.53

(i) *Prevalence*:

The prevalence data according to parasite stage and vector parity are displayed in Fig. 3.3a. As expected, they indicate the absence of L2 and L3 larvae in younger mosquitoes (nulliparous and 1D). Analyses based on a generalised linear model have shown that the infection prevalence varies significantly with parasite stage ($P < 0.05$). The association between infection prevalence and parasite stage in 2D and 3D mosquitoes is shown in figure 3.3a. There is also some evidence of an interaction between mosquito parity and parasite stage which is mainly due to a relatively low prevalence of L3 infection in 2D mosquitoes (again, this probably reflects the impact of developmental time delays).

(ii) *Intensity and overdispersion*:

Since the large number of zero counts made all comparisons between intensity distributions significant, we adopted the more conservative procedure of assessing the differences between the positive counts only by chi squared test. The mean parasite intensity in different parous mosquitoes is displayed in Fig. 3.3b. They indicate a convexity in the age-intensity

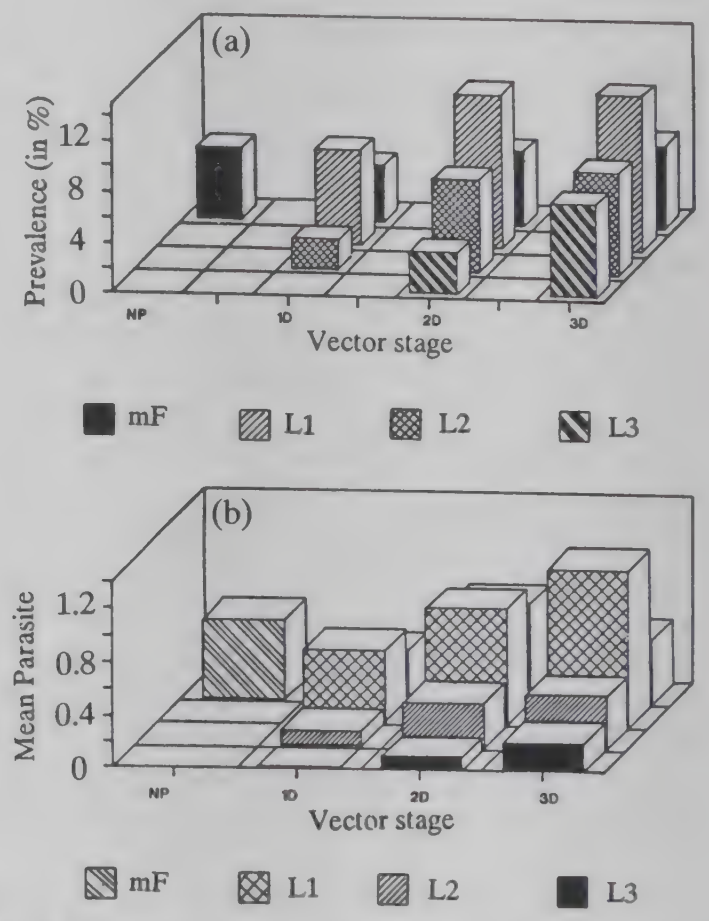


Fig. 3.3 Observed prevalence (a) and intensity (b) of infection in relation to mosquito parity.

relationship, with a peak in the mean L1 larval count in 3D mosquitoes, which subsequently declines in older parasite and vector stages. Another significant feature of the intensity comparison is that, overall, the mf counts do not show a strong trend of heterogeneity between stages. This is significant, since (as for the prevalence analysis) it indicates that the pattern of recent infection in the older vector stages is not affected by the presence of later parasite stages in older mosquitoes. This is also apparent from the variance/mean ratios shown in Table 3.1.

Although the variance/mean ratio peaks in the 2D mf count, it also remains high in all the other mf counts. The degree of overdispersion then falls through all

parasite stages, particularly in the 3D vector stage. Overall, parasite stage seems to be more important than vector stage in determining the decline in overdispersion with the host's experience of infection. However, this effect is the strongest in the older vector stages, indicating that this variable should also be considered.

The main finding of this analysis is that the distribution of parasite infection in vectors of lymphatic filariasis (as measured by mf counts) can be described by the same statistical model as that proposed for blood sampling mf in the human population. In particular, the mf count distribution in nulliparous mosquitoes is remarkably well described by the (zero-truncated) mixed Poisson distribution used to fit the equivalent pattern of mf counts in humans. Since the average blood volume taken by the vector is significantly less than that used for finger prick blood sampling, we might expect the mosquito distribution to have a lower mean and be more overdispersed. *The similarity of the observed vector and human distributions therefore indicates either that counts from mosquito dissections reflect a higher sampling efficiency, or that there may be some concentration of parasites by the vector. The overdispersion analysis of Table 3.1 also confirms the finding that parasite distributions become less overdispersed with parasite stage, reflecting a lower proportion of vectors with high parasite loads. This provides evidence of density dependence in the host-parasite relationship, either in parasite survival or parasite-induced host mortality.*

The analysis of infection prevalence and intensity patterns indicate a convexity in their relationship with parasite stage; again this may be a reflection of density dependent mechanisms such as parasite induced host mortality. *These results also indicate that vector stage may be less important in determining observed patterns, although the degree of overdispersion of infection intensity does vary to some extent with vector parity*. A future analysis will consider these distributions explicitly, particularly in order to investigate how they reflect density dependent processes. Further analysis would be necessary to describe the overall dynamics of infection in human host and transmission in the vector population.

3.1.2. **Post Control Epidemiological Evaluation:**

The Integrated Vector Management (IVM) programme demonstrated by the Vector Control Research Centre achieved significant reduction in vector density. However, this gain could not be sustained after switching over from IVM to conventional methods. The resurgence of the vector population was rapid in the IVM areas whereas in the comparison area, the vector population remained more or less stable. This study examines the impact of vector resilence on epidemiological parameters, after withdrawal of IVM programme in Pondicherry. Though the epidemiological impact of vector control programmes has been assessed in many such pilot projects during the implementation stage, the impact of vector resilience after the withdrawal of programmes against lymphatic filariasis has hitherto not been studied. Such post control epidemiological evaluation is an important aspect of the epidemiology of a parasitic disease which provides information not only on the re-establishment of disease, but also indicates the duration of control period for complete elimination of a long living parasite like *Wuchereria bancrofti*. This study examinines the epidemiological impact of the resurgence of the vector population.

A mass blood survey was conducted from January to June, 1989 to detect the microfilariae in peripheral blood by the finger prick method. The sampling design followed in the survey was similar to earlier surveys carried out in 1981 and 1986. The samples were age-stratified and weighted according to the demography of the area with a minimum target of 5% sample in each age class. The sample also included persons who were covered in earlier surveys. Blood smears were collected from 30,813 persons (9.15 % of the estimated total population of 336,604) in 1989. The sampling distribution over different age classes was similar to that in the previous two surveys carried out in 1981 and 1986. Even in this survey, a target minimum of 5% sampling was achieved in all age classes except in the 0-3 age class (Table.3.2) due to practical constraints. Entomological parameters based on resting and biting collections were also monitored continuously after the withdrawal of control operations at fortnightly intervals in two sites, one from each area.

(a) *Effects on the Entomological parameters*:

The effect of switching from IVM to conventional vector control on resting and biting density of *Cx.quinquefasciatus* is illustrated in Figs. 3.4a and 3.4b. While resting density increased steadily in the IVM area after 1986, this parameter, after an initial

Table:3.2

Age structure and sampling of population in the blood survey in 1989

Age (years)	Population Size	% of total population	Sample size	% of total sampled	% covered in each age class
0-3	29789	8.9	798	2.59	2.68
4-5	19860	5.9	1068	3.47	5.38
6-10	49649	14.8	3833	12.44	7.72
11-15	42076	12.5	4806	15.60	11.42
16-20	29285	8.7	4234	13.74	14.46
21-25	26592	7.9	3245	10.53	12.20
26-30	24909	7.4	2938	9.53	11.80
31-40	42412	12.6	4280	13.89	10.09
41-50	31641	9.4	2646	8.59	8.36
> 50	40392	12.0	2965	9.62	7.34
TOTAL	336604	100	30813	100	9.15

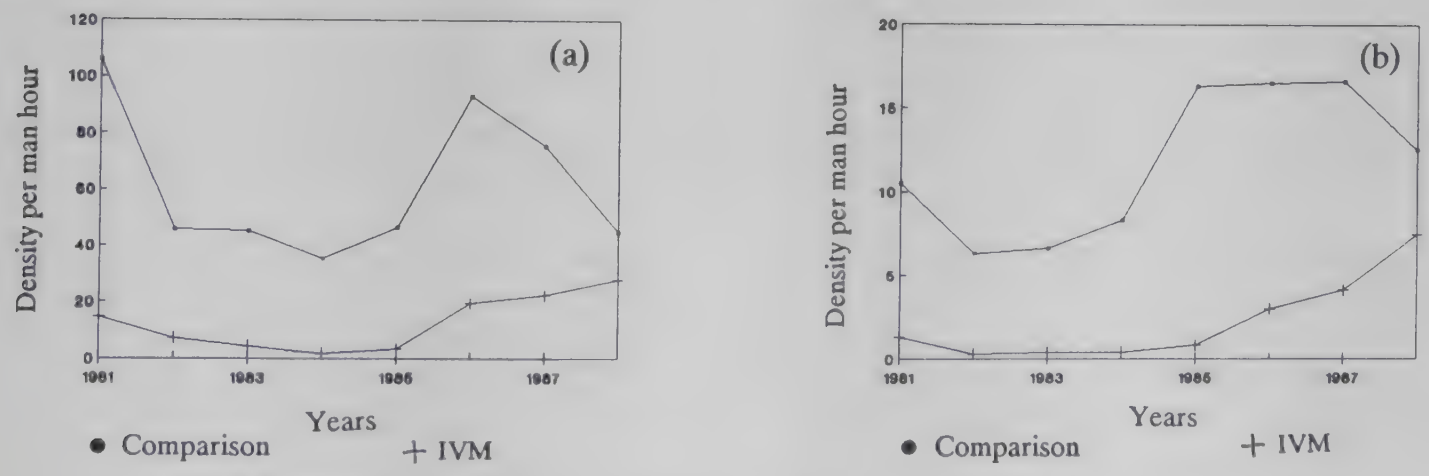


Fig. 3.4. Indoor resting (a) and night biting (b) density of *Cx. quinquefasciatus* in IVM and comparison areas.

increase declined in the comparison area. In the comparison area, biting density started declining after 1987, whereas in the IVM area it continued to increase. However, in the IVM area both resting and biting population were lower than the comparison area during the study period.

The effect of withdrawal of control operations is also measured in terms of the Annual Transmission Index (ATI) and these are depicted in Fig. 3.5 for both IVM and comparison areas. While this parameter showed an increasing trend in the comparison area from 1981 to 1986, it declined in the IVM area for the same

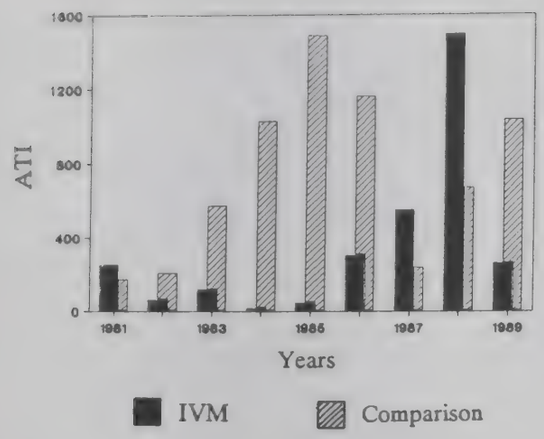


Fig. 3.5 Annual transmission index in comparison and IVM areas.

period. However, after withdrawal of control operations in the IVM area this began to increase from 1986 to 1988. But in comparison area this parameter declined in 1987 and then started increasing.

(b) *Effects on the Parasitological Parameters*:

(i) *Age prevalence and Intensity*:

The age prevalence of microfilaraemia in 1981,1986 and 1989 for comparison and IVM areas are shown in Figs. 3.6a & 3.6b. The age profiles in all surveys in the two areas remained qualitatively same but differed quantitatively. In both areas, mF prevalence continues to show a declining trend in all the age classes. But it showed a marginal increase in 1989 compared to 1986 in the IVM area over the age class of 0-5 years. However, the difference observed in the 4-5 age class was not statistically significant (Table 3.3), (in the 0-3 age class a test of significance could not be carried out because in earlier surveys microfilariae was not detected). While the separation of age prevalence profiles was well marked in all age classes in the IVM area, the same was observed only in older age classes (above 25) of the comparison area between 1981 and 1986. In IVM area all age classes except 0-5 age class showed a declining trend between 1986 and 1989. However in the comparison area, the decline was only upto the age class 20. The observed decline in prevalences from 1986 to 1989 was found to be significant over the age class 6-15 years in both IVM and comparison areas and also in the 26-30 age class of the IVM area. Statistical analysis of the relative change in the prevalence of infection between the two areas for each age class indicated that the prevalence in the IVM area declined significantly only in the 26-30 age class (Table.3.3).

The age-intensity of infection for comparison and IVM areas are shown in Figs. 3.7a & 3.7b. The intensity increases from 0 to 20 years of age and then began declining in adults. Comparison of the age intensity profiles shows that in the IVM area the decline in infection intensity between 1981 and 1986 was more marked in almost all age classes than that between 1986 and 1989, when a marginal decline was observed only in some of the adult age classes. In contrast, the age intensity profiles in the comparison area appear similar to each other in adult age classes and in older age classes a substantial increase was observed from 1986 to 1989. Comparison of the microfilarial densities in 1986 and 1989 did not reveal (using non-parametric Mann-Whitney U-test) any significant difference for any age class in either area.

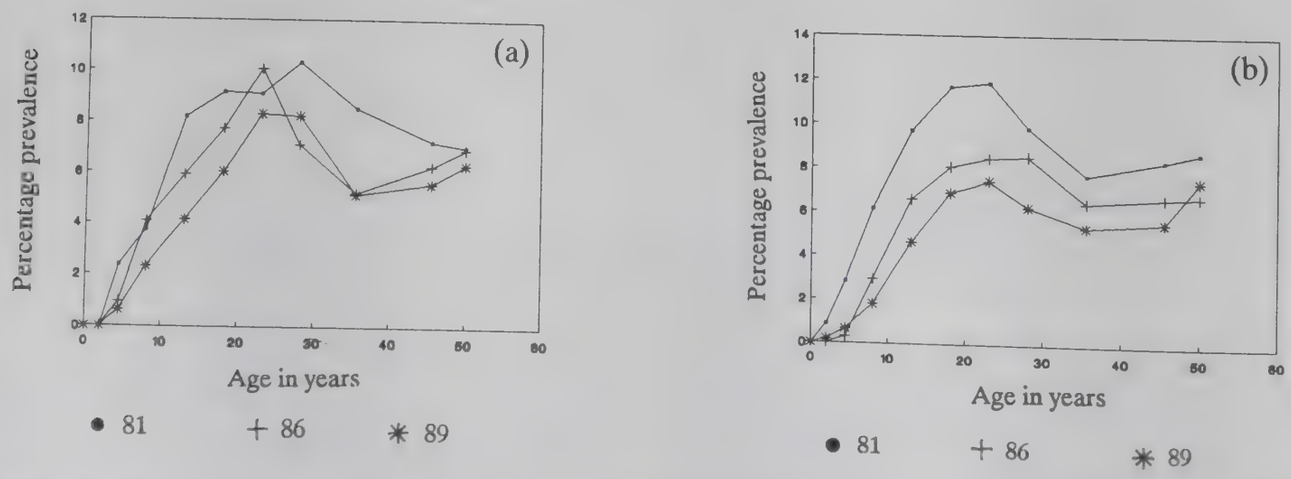


Fig. 3.6 Age prevalence of microfilaraemia in comparison (a) and IVM (b) areas in 1981, 1986 and 1989.

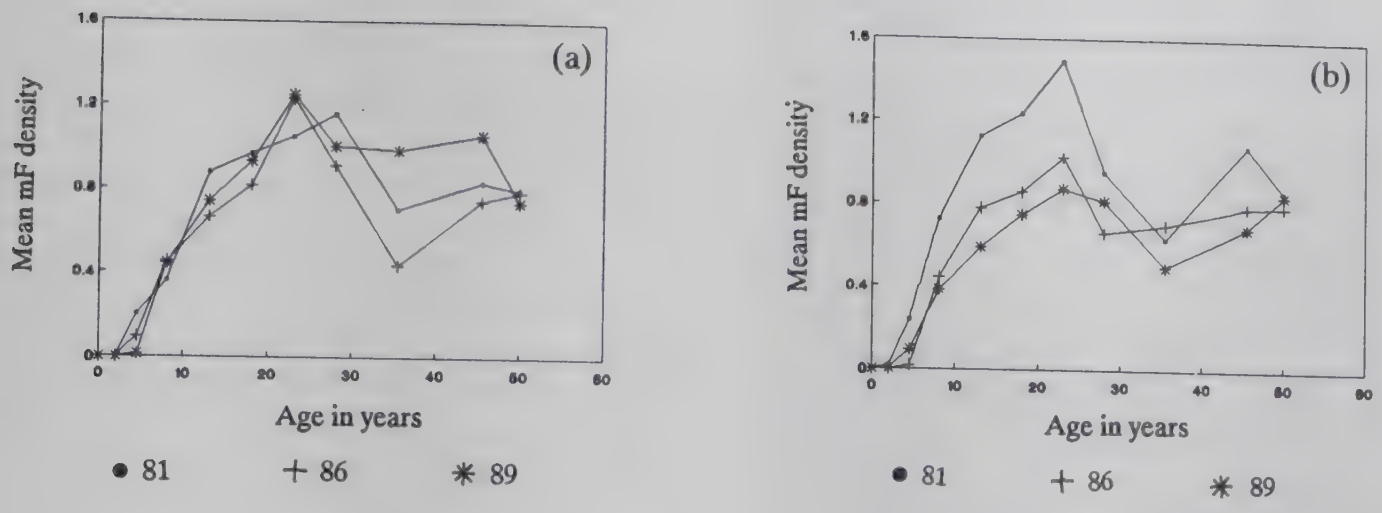


Fig. 3.7 Age specific intensity of microfilaraemia in comparison (a) and IVM (b) areas.

Table 3.3.

Age specific microfilaraemia prevalence in Comparison and IVM Areas.

Age (Years)	Comparison area			IVM area			Relative Change Odds ratio : p
	86	89	Chi.Sq. Prob.	86	89	Chi.Sq. Prob.	
1-3	0.00	0.00	-	0.00	0.18	-	-
4-5	0.94	0.63	0.64	0.31	0.66	0.29	0.3104
6-10	4.09	2.31	0.01*	2.94	1.80	0.01*	0.7667
11-15	5.92	4.14	0.02*	6.60	4.66	0.00*	0.9652
16-20	7.72	6.03	0.07	8.10	6.90	0.08	0.6001
21-25	10.06	8.29	0.13	8.49	7.46	0.19	0.6804
26-30	7.09	8.22	0.33	8.56	6.27	0.01*	0.0147
31-40	5.20	5.16	0.95	6.49	5.39	0.07	0.3284
41-50	6.27	5.57	0.50	6.72	5.60	0.17	0.7782
> 50	6.93	6.33	0.54	6.78	7.49	0.41	0.3184
Overall	6.33	5.32	0.00*	6.36	5.21	0.00*	0.7100

* Denotes the significance at 0.05 level.

(ii) *Prevalence and Intensity among resurveyed people*:

Out of 30,813 persons sampled in 1989, 9,592 persons were recruited once in an earlier survey also either in 1981 or in 1986 and 3682 were surveyed both in 1981 and 1986. Since mF positive people in earlier surveys have received chemotherapy in 1986, it was felt that the mF rate and mF count might have been influenced by chemotherapy instituted by VCRC. Therefore, mF rate and mF density in these three groups were compared separately. An analysis of these comparisons, using the age-prevalence data, is presented in Table 3.4. This shows that the prevalence in resurveyed people was not significantly different from those who were not recruited in earlier surveys.

However, an age-wise analysis indicated that the prevalence in some age classes over 15 years of age was significantly lower in resurveyed individuals than in those who had not been surveyed earlier in the IVM area (Table-3.4). By contrast, in the comparison area, the age specific prevalences were unaffected by the number of times individuals were recruited in earlier surveys except for the 41-50 age class. These differences between IVM and Comparison areas probably reflect the impact of post-sampling chemotherapy in the former.

An overall decline in mf intensity was observed in both comparison and IVM areas between freshly sur

Table 3.4.

Comparison of age specific microfilaraemia prevalence between freshly surveyed and resurveyed people in 1989.

Age (Years)	Comparison area Surveyed			IVM area Surveyed		
	Once n = 5859	Twice 3239	Thrice 1299	Once 11680	Twice 6353	Thrice 2383
1-3	0.00	0.00	0.00	0.18	0.00	0.00
4-5	0.66	.0.00	.0.00	0.69	0.00	0.00
6-10	2.47	0.00	1.96	1.96	0.00	1.45
11-15	4.59	2.81	3.83	5.38	4.11	3.81
16-20	7.09	3.70	5.42	9.16	4.55*	4.58*
21-25	9.61	6.57	6.65	7.84	8.73	6.14
26-30	8.01	6.73	9.26	7.10	4.39	5.22
31-40	4.70	4.35	6.13	6.83	2.25*	4.35*
41-50	7.66	2.84	4.48*	7.31	3.57*	4.73*
>50	7.79	4.28	5.76	8.74	5.64	6.95
Overall	5.58	5.28	4.23	5.75	4.44	4.57

* Denotes the significance of prevalence at 0.05 level.

veyed and resurveyed people. A comparison of mf densities of freshly surveyed with resurveyed people based on non-parametric (Mann-Whitney) tests revealed significant differences between freshly surveyed and resurveyed group indicating the reduction in density among the resurveyed people.

(iii) *Loss and gain of infections*:

Since the prevalence reflects both the loss and gain rates of infection, these rates were estimated for both the areas and for two periods. The analysis of the resurvey data showed that the overall rate of acquisition increased marginally from 0.0126 per year (in 1981-86) to 0.0142 per year (in 1986-89) in the IVM area after the control methodology was changed. In the comparison area also the overall rate of acquisition increased from 0.0094 per year to 0.0158 per year for the same period. While the age specific rate of acquisition was generally unchanged in the IVM area, an increase in all age classes was observed in the comparison area (Figs. 3.8a & 3.8b). The increase was

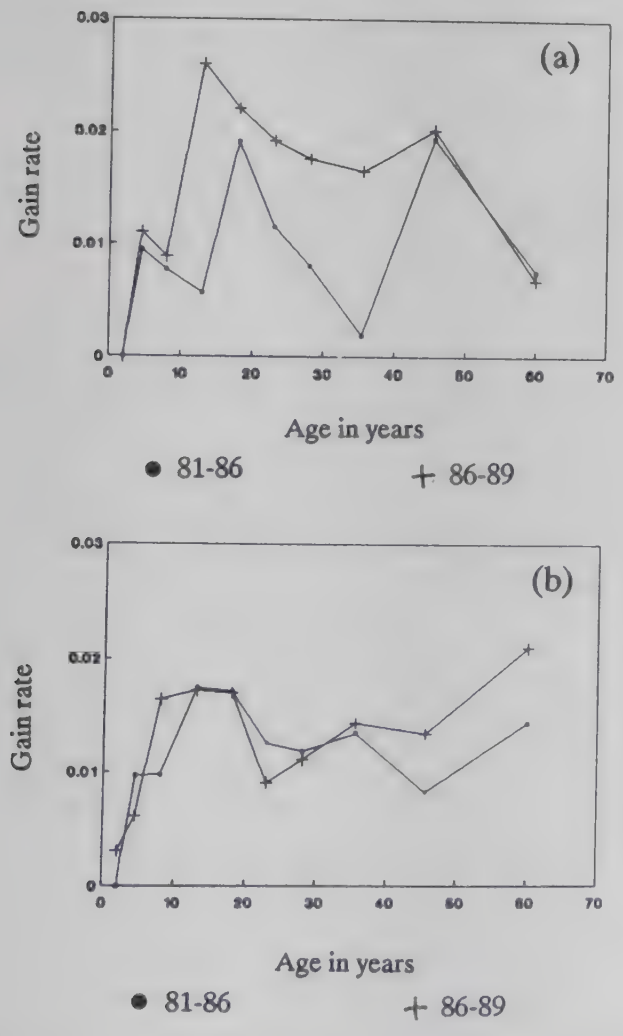


Fig. 3.8 Age specific rate of acquisition of infection in comparison (a) and IVM (b) areas.

substantial in the age classes over 11 years. The overall loss rate increased from 0.2150 to 0.361 per year in comparison area, and 0.196 to 0.409 per year in the IVM area. Figs. 3.9a and 3.9b show the age specific

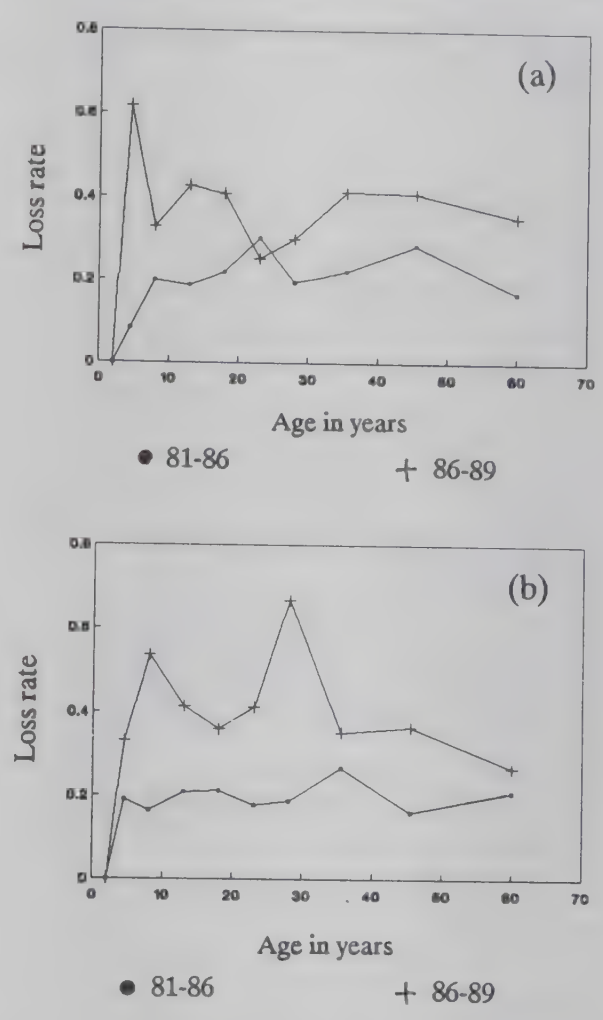


Fig. 3.9 Age specific rate of loss of infection in comparison (a) and IVM (b) areas.

rate of loss of infection in comparison and IVM areas. In both areas, the loss of infection was higher than that of gain of infection in all age classes of 1986-89 when compared to 1981-86.

In summary, even though the vector density significantly increased in the IVM areas, its impact on overall epidemiological parameters was not clear. This is probably due to the fact that changes in the parasite population are much slower than expected. In terms of future work, it would be worth to analyse if there are any spatial variations within the IVM and comparison areas.

3.2. CLINICAL EPIDEMIOLOGY:

The clinical outcome of filariasis infection depends upon the complex interaction of several factors re-

lated to human host, parasite species and environmental conditions. Therefore attempts were made to understand and define the dynamics of these interactions by using appropriate analytical tools.

3.2.1. Clinical manifestations: effect of age and gender

The effect of age and gender on the clinical consequences of filariasis infection was analysed. The data from the door to door clinical and parasitological surveys done in Pondicherry in 1986 were utilized. In the clinical survey, a detailed physical examination was done and clinical history was recorded. The samples in clinical and parasitological surveys were age stratified, and weighted according to demography of the population, with a target minimum of 1.5% and 5% in each age class in the clinical and microfilarial surveys, respectively.

A total of 6,493 persons (2.6% of population) was examined for disease in the clinical survey. The age specific coverage exceeded the target sample in the survey. Details of parasite survey are provided elsewhere (chapter: 3.1). The prevalence of disease and and microfilaraemia was found to be 6.59% and 6.34%, respectively. The prevalence of disease was significantly higher in males (13.7%) than in females (2.3%) ($P < 0.001$). The prevalence of microfilaraemia was 7.14% and 5.59% in males and females, respectively ($P < 0.05$ for difference between sexes). Hydrocele (11.88%) was the most prevalent condition in males and lymphoedema (2.11%) in females. The prevalence of lymphangitis was 0.17% in females and 0.89% in males ($P < 0.05$ between sexes).

The comparative age-prevalence of disease and microfilaraemia for males and females is shown in Figs. 3.10a and b. The prevalence of disease in males was clearly age dependent and steadily increased from the 9-10 years age class. Disease prevalence in females was low until about 20 years, following which it increased until the oldest age class, but at a much slower rate than in males. Unlike disease, the age specific pattern of microfilaria prevalence in both sexes was similar. Prevalence of disease was significantly higher in males than in females from 15 years of age onwards (Table 3.5). The difference between the sexes was entirely due to the high prevalence of hydrocele in males. The age specific prevalence patterns of chronic irreversible manifes

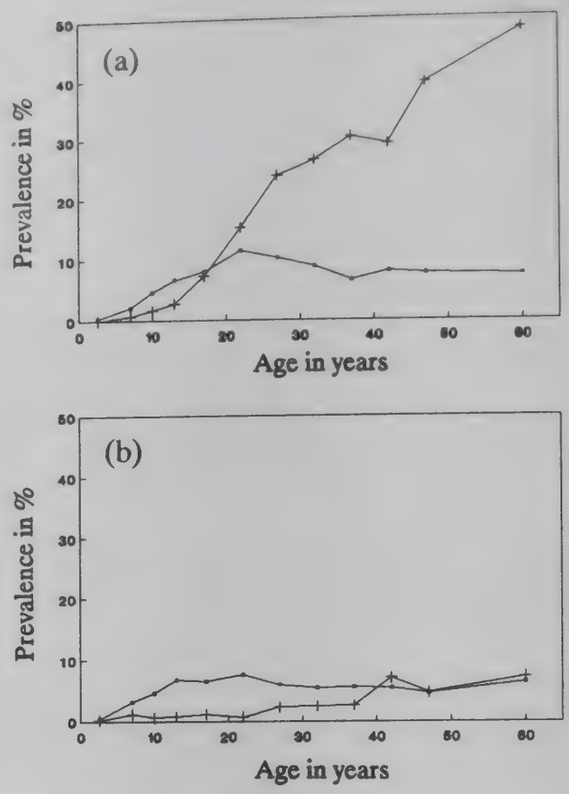


Fig. 3.10 Age specific prevalence of disease (+) and microfilaraemia (•) in (a) males and (b) females.

tations showed marked age dependency (Fig. 3.11a), but those of acute episodic signs (Fig. 3.11b) were independent of age.

An attempt was also made to examine the claim that gender dependent clinical expression differs between North and South Indian populations. Data on the point prevalence of disease in males and females was compared to examine the relationship between the

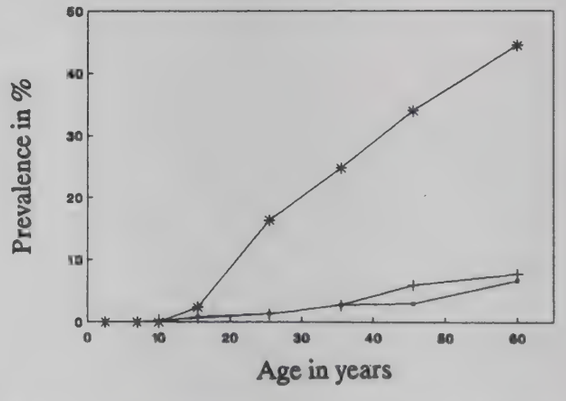


Fig. 3.11a Age specific prevalence of chronic lymphoedema for females (•) and males (+) and hydrocele (*).

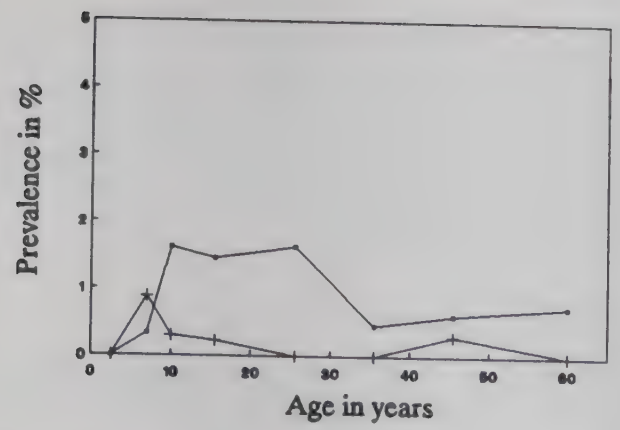


Fig. 3.11b **Age specific prevalence of acute episodic lymphangitis for females (+) and males (•).**

sexes, and the differences between North and South Indian populations. Data with sample age structure for both sexes were available for 12 localities (Patna,Bhagalpur, Calcutta, Trichur, Mangalore, Ponani, East Godavari, 2 villages in Puri, rural and urban Monghyr and Pondicherry), which were therefore used in the subsequent analyses. Since the sample structure differed between the sexes, the point prevalence of disease in females in each area was computed by standardizing against the sample structure for males using established techniques. The results (Fig. 3.12) showed that there was a significant

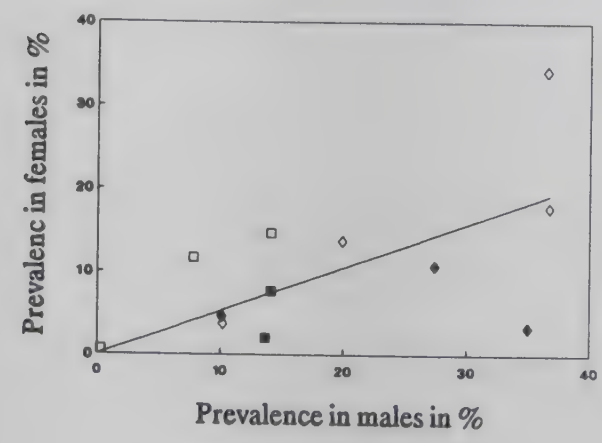


Fig. 3.12 **Relationship of point prevalence of disease between males and females in North (◊) and South (□) India. (Darkened symbols represent studies where detailed clinical examinations were carried out).**

Table 3.5

Age and gender structure of sample in clinical and parasitological surveys, and comparison of disease and infection prevalence between sexes.

Age Class (years)	Clinical Survey Sample size Male	Female	Chi Square p Value#	Parasite Survey Sample Size Male	Female	Chi Square p Value#
0-5	423	457	0.519	1086	937	0.419
6-8	295	339	0.410	1184	1075	0.151
9-10	247	330	0.219	1386	1379	0.976
11-15	262	375	0.062	1705	1656	0.930
16-20	289	505	<0.001*	2471	2494	0.065
22-25	171	421	<0.001*	1988	1982	<0.001*
26-30	134	351	<0.001*	1548	1845	<0.001*
31-35	121	243	<0.001*	1231	1444	<0.001*
36-40	89	233	<0.001*	975	1292	0.381
41-45	86	184	<0.001*	741	942	<0.050*
46-50	79	150	<0.001*	660	819	<0.050*
>50	270	439	<0.001*	1838	1937	0.226
TOTAL	2466	4027	<0.001*	16813	17802	<0.050*

* : Significant difference in proportion
# : Males Vs. Females

correlation between the occurrence of disease and gender ($r = 0.59$, $P < 0.05$), but this relationship did not significantly differ between North and South Indian populations (comparison of regression coefficients between two regions was not significant; $P > 0.05$). The point prevalence of disease was generally lower in females (by a proportion of 0.39) than in males. Details of clinical examination procedures were available only for 4 studies covering 5 areas (East Godavari, Calcutta, 2 villages in Puri and Pondicherry). The relationship of disease prevalence between sexes was clearly evident in these areas (Fig. 3.12).

The study indicated that the age structure of a survey sample crucially determines the apparent prevalence of chronic disease. The age dependency of chronic signs appears to be due to the accumulation of chronic cases in the population. The study also indicated that the gender dependency of clinical manifestations is primarily due to the occurrence of hydrocele in males. Failure to appreciate the importance of gender and age to disease prevalence estimation appears to have led to a misconception about disease patterns in India. Most of the earlier surveys were carried out primarily to identify filariasis endemic areas and the clinical surveys were secondary and therefore, often superficial. This must inevitably lead to underestimation of the prevalence of hydrocele, the clinical sign which crucially determines the difference in prevalence between the sexes. These results suggest that the claimed difference between disease patterns in North and South Indian populations is attributable to the difference in methods of clinical examination and recording.

3.2.2. **Dynamics of infection and disease:**

Although the major clinical manifestations of bancroftian filariasis have been extensively described, their relationship with the course of infection remains enigmatic. There are several difficulties in studying this relationship: appearance of disease is a slow process, most microfilaraemic persons are asymptomatic and most symptomatic individuals are amicrofilaraemic at any given point of time. The recent work in this area has sought correlates between disease and immunological status. It has been suggested that there is a spectrum of disease with different manifestations corresponding to clusters of patients (perhaps predisposed genetically) with different immune responses. To study the dynamics of infection and disease, two approaches were made: (A) real situation analyses using the data available from door to door surveys and people examined at the VCRC filariasis clinic and (B) application of mathematical models using the clinical and parasitological data bases.

(A) Real situation analyses:

The following three groups were identified for the analyses:

Group -I: 3,170 randomly selected persons examined clinically and also for microfilaraemia in 1986 by door to door visits, constituted this group. The data from this group was analysed for prevalence of disease in relation to microfilaraemia.

Group -II: 1,103 microfilaria carriers who were examined clinically at the VCRC filariasis clinic formed the second group for detailed analyses.

Group -III: 1,024 persons examined for microfilaraemia both in 1981 and 1986 (out of 7,525 persons covered both the surveys) and examined clinically at the filariasis clinic in 1986 formed the third group. The data from this group was analysed for clinical outcome in relation to change in mF status over the 5 year period (1981 to 1986).

Of the 3,170 persons examined in group - I, 201 (6.34%) were microfilaraemic and 432 (13.6%) were diseased. Clinical manifestations of filariasis were recorded in 27 microfilaria (mF) carriers (13.43%) and in 405 (13.64%) amicrofilaraemic persons ($\chi^2 = 0.005$: $P = 0.98$, between the two groups). Comparison of clinical spectrum between the two groups is shown in Table 3.6.

Comparison of age specific patterns of disease prevalence between mF carriers and amicrofilaraemic persons (Fig. 3.13) showed a clear age dependant rise in both groups and there was no significant difference between the two in any age class ($P > 0.05$). Of the 1,103 microfilaria carriers in group II, 173 (15.68%) had filarial disease manifestations and there was no significant difference between the disease rate in this group and the mF carriers of group I ($\chi^2 = 0.50$, $P = 0.47$). The disease rate in females (11.17%) was significantly lower ($\chi^2 = 15.45$, $P = 0.0001$) compared to males (19.96%). Comparison of clinical spectrum between the sexes

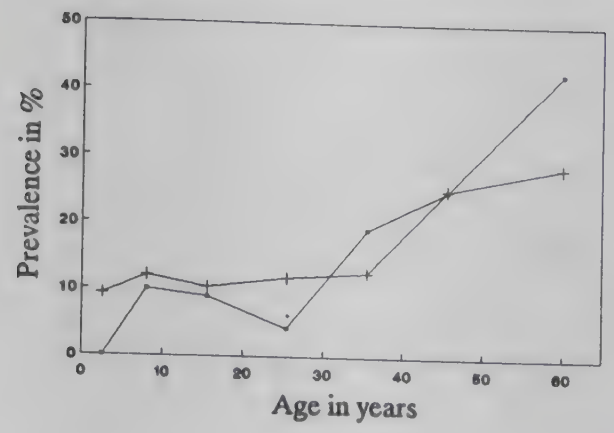


Fig. 3.13 Comparison of age specific prevalence of disease in microfilaraemic (•) and amicrofilaraemic (+) population of Pondicherry (Group I).

Table 3.6

Comparison of clinical manifestations between microfilaraemics and amicrofilaraemics in Pondicherry (Group I).

Clinical manifest-ations	Microfilaraemia status: mF +ve (n = 201) No. +ve(% of n)		mF -ve (n = 2969) No. +ve(% of n)		Test of significance (p Value**)
SPECIFIC:					
A. Acute:					
1. Filarial fever	8	(3.98)	213	(7.17)	0.11
2. Lymphangitis	1	(0.50)	19	(0.64)	0.63
3. Funiculitis	1	(1.19)*	12	(1.18)*	0.63
4. Epididymo-orchitis	3	(3.57)*	19	(1.86)*	0.22
B. Chronic:					
1. Hydrocele	15	(17.86)*	112	(10.97)*	0.08
2. Lymphoedema	1	(0.50)	54	(1.82)	0.13
3. Lymph varix	2	(1.00)	2	(0.07)	0.02***
4. Lymph scrotum	0	(9.00)*	3	(0.10)	0.78
5. Chyluria	6	(2.99)#	34	(1.15)	0.05
NON SPECIFIC:					
Lympadenitis:	17	(8.46)	188	(6.33)	0.30

* : only males were considered; ** Chi square/Fisher's exact test done.
*** : Difference significant; # By history

(Table 3.7) showed significant difference in occurrence of lymphoedema and lymphadenitis. Age specific patterns of disease rate (Fig. 3.14) were similar in both sexes until young adult age classes (up to about 30 years) showing a monotonic rise, following which there was clear separation. Significant difference in disease rates between sexes was observed in age classes beyond 40 years only ($P < 0.05$). Age specific patterns of individual manifestations (Fig. 3.15) showed that the occurrence of lymphangitis was high in children. The age dependent monotonic increase in occurrence of hydrocele was most distinct. Appearance of lymphoedema in females was relatively earlier compared to males (Figure 3.15, Table 3.7). There was no association between microfilarial density and clinical outcome (Table 3.8) in either sex

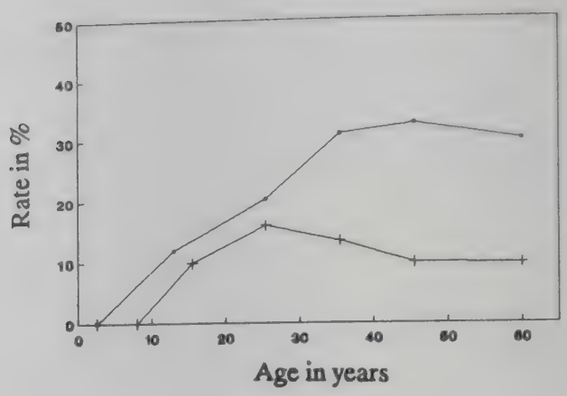


Fig. 3.14 Comparison of disease rate between female (+) and male (•) microfilaria carriers in Pondicherry. (Group: II).

Table 3.7

Comparison of clinical manifestations between microfilaraemic females and males (Group II).

Clinical Manifestations	Males n = 566 (% of n)	Females n = 537 (% of n)	p Value (*)	Age (Years) Range	Median	mF count Range	Median
SPECIFIC							
A. Acute							
Filarial Fever	26(4.6)	16(3.0)	0.21	7-58	24.0	1-45	3.5
Lymphangitis	14(2.5)	17(3.2)	0.61	6-60	19.0	1-68	4.0
Funiculitis	6(1.1)	-	-	21-37	23.5	2-46	5.5
Epididymo Orchitis	11(1.9)	-	-	15-46	32.0	1-46	6.0
B. Chronic							
Lymphoedema	4(0.9)	14(2.6)	0.01#	12-74	35.0	1-44	3.0
Hydrocele	58(10.2)	-		15-76	39.5	1-90	3.5
Lymph Varix	3(0.5)	1(0.2)	0.33	24-43	30.0	1-5	2.5
Lymph Scrotum	1(0.2)	-	-	54**	-	29**	-
Chyluria	12(2.1)	15(2.8)	0.65	12-74	30.0	1-49	8.0
NONSPECIFIC							
Lymphadenitis	143(25.3)	293(54.6)	0.00#	5-76	20.0	1-211	4.0

* : Significant difference between males and females done using Chi square/Fisher's exact test.
** : Single case
# : Difference significant.

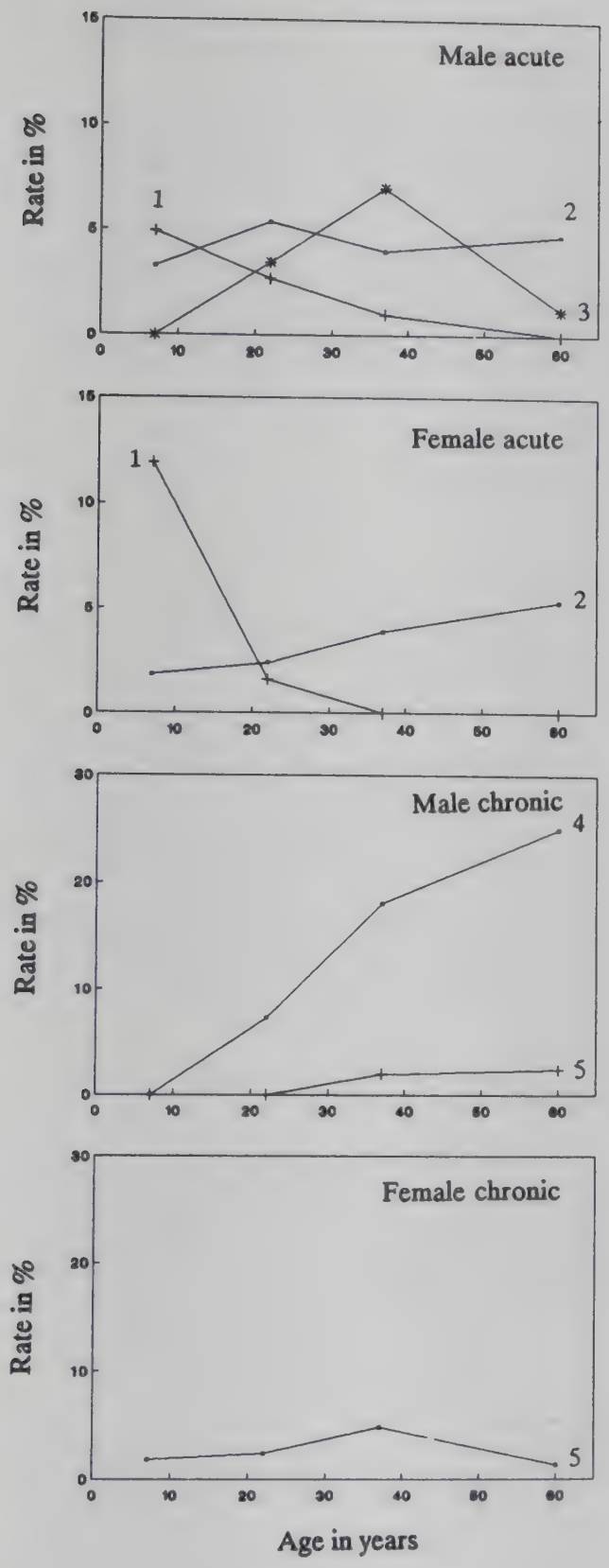


Fig. 3.15 Comparison of age specific patterns of acute and chronic manifestations in microfilaria carriers: Group II (1: Lymphangitis, 2: Filarial fever, 3: Funiculitis/Epididymo-orchitis, 4: Hydrocele and 5: Lymphoedema).

(males: Z = 0.18, P = 0.43; females: Z = 0.49, P = 0.31). The mean microfilaria counts in different age classes did not show any significant difference between the symptomatic and asymptomatic individuals (Table 3.9). There was no significant difference (P > 0.05) in the occurrence of any individual manifestations (Table 3.10).

Table 3.8

Distribution of symptomatic and asymptomatic mF carriers according to mF count (Group II).

mF counts	Symptomatic Male	Symptomatic Female	Asymptomatic Male	Asymptomatic Female
< = 10	85	45	328	353
11-20	12	10	66	64
21-30	5	2	25	30
31-40	3	1	9	14
> = 41	8	2	25	16
Total	113	60	453	477

According to the mF status in 1981 and 1986, the 1,024 persons in group III were classified into 4 sub groups: (a) mF +ve both in 1981 and 1986 (n = 155), (b) mF +ve in 1981 but -ve in 1986 (n = 138), (c) mF -ve both in 1981 and 1986 (n = 613), and (d) mF -ve in 1981 but mF +ve in 1986 (n = 118). In these subgroups, 12.26%, 12.32%, 12.39% and 14.41% respectively had developed clinical manifestations within the five year period. Comparison of occurrence of disease between sub group 'c' versus other sub groups did not reveal any significant difference in disease rate (c versus a: χ^2 = 0.007, P = 0.92; c versus b: χ^2 = 0.01, P = 0.91; c versus d: χ^2 = 0.20, P = 0.65). In the 4 sub groups the acute disease rate was 3.23%, 5.80%, 4.89% and 5.36%, and, the chronic disease rate was 9.03%, 5.8%, 7.50% and 8.47% respectively. There was no significant difference either in acute or in chronic disease rate between sub group 'c' versus others (P > 0.05). Application of Log odds ratio test for above analyses also did not show any significant difference for total/ acute/ chronic disease (Z < 1.96; P > 0.05). The results indicated that the prevalence of disease was independent of mF status at a given point of time. Further, the changes in mF status over a 5 year period did not influence the occurrence of disease. However, the possible role of mF in the pathogenesis of chronic manifestations cannot be ruled out.

Table 3.9

Mean microfilarial counts among symptomatic and asymptomatic carriers (Group II).

Age Class (in yrs)	Male		Female	
	Symptomatic mean±s.e(mean)	Asymptomatic mean±s.e(mean)	Symptomatic mean±s.e(mean)	Asymptomatic mean±s.e(mean)
0 - 14	12.73 ± 5.82	10.32 ± 1.43	10.80 ± 2.99	13.38 ± 1.42
15 - 29	10.17 ± 2.57	9.73 ± 1.10	7.55 ± 2.07	8.95 ± 1.17
30 - 44	11.07 ± 3.24	11.49 ± 2.05	6.14 ± 1.53	8.33 ± 1.29
> =45	10.31 ± 2.60	11.03 ± 1.96	8.43 ± 4.34	7.50 ± 1.23
Total	10.69 ± 1.56	10.31 ± 0.74	7.87 ± 1.26	9.55 ± 0.75

Comparison of mean mFc between symptomatic and asymptomatic individuals for both sexes in each age class was not significant. ($p > 0.05$, t - independent test).

Table 3.10

Proportion (%) of mF carriers positive for different clinical manifestations in relation to mF count (Group II).

mF counts	Sample Size	Filarial Fever	Lymphan -gitis	Funicu -litis	Epididymo -orchitis	Hydro -cele	Lymph -oedema	Chyluria
< =10	811	4.19	2.71	0.97	1.94	11.14	1.60	2.22
11-20	152	3.29	3.29	1.28	2.56	5.13	1.97	2.63
21-30	62	3.23	3.23	0.00	0.00	3.33	0.00	1.61
31-40	27	0.00	3.70	0.00	0.00	16.67	3.70	0.00
> =41	51	1.96	1.96	3.03	3.03	15.15	1.96	7.84
Total	1103	3.81	2.81	1.06	1.94	10.25	1.63	2.45

(B) Application of mathematical models.

Using mathematical models a dynamic perception of the development of lymphatic disease was explored, in which it was hypothesized that individuals progress from one category to another during the development of pathology. It was assumed that individuals progress from uninfected to asymptomatic microfilaraemic, and then become amicrofilaraemic as lymphatic pathology develops. This model tested the prediction that the proportion of the population which develops lymphatic disease should be the same as or less than the proportion of microfilaria carriers who subsequently become amicrofilaraemic.

Conceptual framework:

Earlier, a reversible catalytic model was used to describe the dynamics of filariasis transmission in Pondicherry (VCRC annual report, 1989). The equilibrium dynamics of the microfilaria negative i.e. uninfected (U) and microfilaria positive i.e. infected (I) portions of the human population were successfully described by the coupled differential equations, given by

$$dI/dt = -bI + aU \quad \text{.....(1)}$$
$$dU/du = -aU + bI \quad \text{.....(2)}$$

where the instantaneous rate of gain of microfilaraemia 'a' and instantaneous loss of microfilaraemia 'b' were estimated. Using a similar model and assuming that b = 0, the cumulative proportion of people at time 't' who had ever been microfilaria positive (I^*_t) could be estimated from:

$$I^*_t = 1 - \exp(-at) \quad \text{.....(3)}$$

where the rate of gain of microfilarial positivity is assumed to be independent of age. The corresponding relationship for age-specific values of 'a' is given by:

$$I^*_{t2} = 1 - (1 - I^*_{t1}) \exp[-a_{t1}(t_2-t_1)] \quad \text{.....(4)}$$

where: I^*_{t2} is the proportion who have ever been microfilaria positive at time t_2; I^*_{t1} is the proportion at time t_1; and a_{t1} is the rate of gain of microfilarial positivity between t_1 and t_2. For the first age class I^*_{t1} is zero and the relationship therefore, collapses to equation (3).

Thus, provided the age-specific infection parameters are known, it is possible to estimate for any age class the proportion of individuals who have at any time been microfilaria positive. The proportion who have been microfilaraemic but have become amicrofilaraemic (R_t), who are assumed to be at risk of progressing to disease is given by

$$R_t = I^*_t - I_t \quad \text{.....(5)}$$

where I_t is the proportion who are microfilaria positive at time 't', estimated from the solution to equation (1). Age specific values can, however, be iteratively estimated for other areas from age-prevalence data using the solution to equation (1):

$$I_{t2} = [a_t/(a_t+b)\{1-\exp[-(a_t+b)(t_2-t_1)]\} + I_{t1}\exp[-(a_t+b)(t_2-t_1)]] \quad \text{.....(6)}$$

where I_{t2} and I_{t1} are the observed prevalences at ages t2 and t1, respectively, and the rate of loss of microfilarial positivity, 'b', is assumed independent of age and relatively constant for a given parasite species, as appears to be supported by the available data.

The following databases were used for the analyses:

A. For Pondicherry population: (i) The longitudinal parasitological data (1981 and 1986) of 7,525 individuals, and (ii) the cross sectional clinical survey data (1986).
B. For Calcutta population: The cross sectional data on the parasitological and clinical aspects of 400 individuals.

Data for the male and female populations were analysed separately due to the marked gender-dependancy of manifestations in clinical bancroftian filariasis (See 3.2.1).

Estimation of the risk of chronic lymphatic filariasis:

The cumulative percentage of men who had been microfilaria positive but became amicrofilaraemic in Pondicherry was estimated from equations (4) and (5) using direct estimates of the rate of loss and gain of microfilaraemia. This percentage shows a monotonic rise from approximately 10 years of age, and is remarkably similar to the age-prevalence profile of chronic filarial disease (hydrocele and lymphoedema) within the same male population (Fig. 3.16a). Thus the proportion of the male population assumed to be at risk of disease closely approximates the observed proportion with clinical manifestations. No such relationship is observed between 'R' and the age-prevalence of chronic disease (lymphoedema only) in females (Fig. 3.16b).

The sensitivity of the risk factor based on the accuracy in the estimation of the rate parameters was also analysed. It was found that the estimation procedure was robust and it was minimally effected by errors in parameter estimation. The age specific rate of gain of microfilaraemia in the Calcutta male population and the subsequent age profile of 'R' were estimated using the equations (4),(5) and (6). This estimate of the male population at risk approximates the ob-

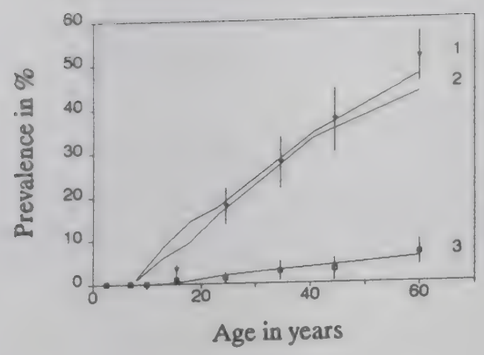


Fig. 3.16a Comparison of prevalence of Bancroftian filariasis with the proportion at risk of developing disease in males in Pondicherry. (1) Proportion of men at risk of disease calculated using equation -5 with age specific rates of acquisition of infection, (2) Proportion of men at risk of disease calculated using equation - 5 with mean rate of acquisition of infection, (3) Proportion of men who are at risk of developing lymphoedema (R* 0.11). The 95% confidence limits are given for prevalence of disease and prevalence of lymphoedema.

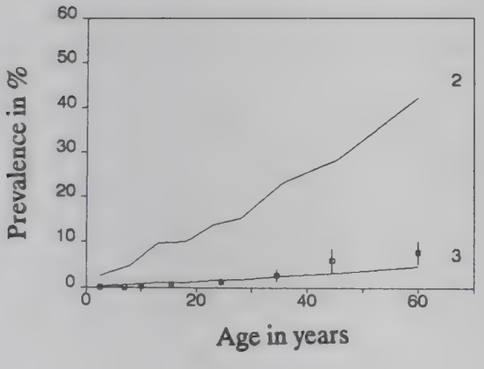


Fig. 3.16b Comparison of prevalence of lymphoedema with the estimated figures in females in Pondicherry, (2) Proporton of women at risk of disease calculated using equation-5 with mean rate of acquisition of infetion, (3) Proportion of women who are at the risk of developing lymphoedema (R*0.11). The 95% confidence limits are given for prevalence of lymphoedema.

served age-prevalence of chronic filariasis (hydrocele and lymphoedema) in men (Fig. 3.17),

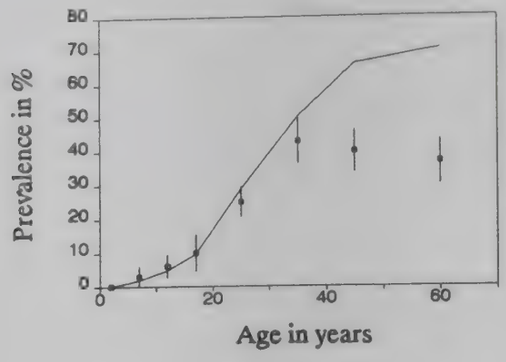


Fig. 3.17 Comparison of prevalence of chronic disease with the proportion at risk calculated from equation-6 for males in Bengal.

though less closely than observed for the Pondicherry data-set (Fig. 3.16a). It is noteworthy, however, that the proportion with observed disease does not exceed the proportion estimated to be at risk of disease.

Estimation of the risk of lymphoedema:

In Pondicherry, the age-prevalence of chronic filariasis in men was linearly related to the age-prevalence of lymphoedema ($r = 0.95$; $P < 0.001$; $DF = 7$). For all age-classes, an average of 11.2% of cases of chronic disease had lymphoedema. Multiplying the age specific values of 'R' by this conversion factor yielded an estimated age-prevalence profile for lymphoedema which closely approximates the observed values for the male population of Pondicherry (Figure 3.16a). However, if 'R' for women is multiplied by the lymphoedema conversion factor (11.2%) for men, this cumulative proportion closely approximates the observed age-prevalence of lymphoedema (Figure: 3.16b). This suggests that the proportion of the population at risk of disease who actually progress to lymphoedema is similar for both sexes. These epidemiological observations are compatible with the immunological hypothesis that clearance of microfilariae from the blood is indicative of enhanced immunological recognition of the parasite which in turn leads to local inflammatory responses, lymphatic damage and obstructive pathology. Although the data show obvious age-dependency in the prevalence of disease, the incidence of

disease is apparently independent of age. This is shown both for the total chronic signs and by the linear relationship between total signs and lymphoedema (Figure 3.16a).

The above analyses suggest that a population exposed to filarial infection can be considered to consist of two sub-populations. In one there is a dynamic sequential progression through four compartments: uninfected; asymptomatic microfilaraemic; asymptomatic amicrofilaraemic; and amicrofilaraemic with irreversible obstructive lymphatic disease. In the last compartment there may be further subdivisions dependent on gender and susceptibility to disease, but not related to age-dependent exposure. In the other section are the individuals who exhibit no signs of infection or disease. These might be assigned to the immunological category of "endemic normal" but equally may simply be uninfected.

Thus, while real situation analyses showed that there is no direct relation of microfilaria status with disease at any given point of time, application of mathematical models suggests that course of events from infection to disease is sequential. However, the dynamics of this disease being complex, disentangling these will require simultaneous clinical, parasitological and immunological study of the same population.

3.2.3. Relation between ABO blood groups and infection and clinical status:

Preliminary investigations carried out at the VCRC had suggested that ABO blood group status did not influence the infection status. However, microfilaria carriers with blood group 'B' had a significantly higher and with group 'AB' had significantly lower probability of developing clinical manifestations (VCRC annual report, 1986-87). Hence, further studies were carried out and data from 1,444 individuals were analysed to determine the relationship between blood group and clinical status.

The blood group distribution of the 1,444 persons according to microfilaria and clinical status is shown in Tables 3.11a, b and c. Chi square statistics did not reveal any significant association between blood group and infection/ clinical status (either alone or in combination). In another approach, it was assumed that the factors influencing infection were blood group, age and clinical status, and similarly, those influencing disease were blood group, age and infection status. In order to identify the important factors among the assumed ones, a logistic model was fitted and the significance of each factor was studied. Advanced statistical GLIM package was used for fitting

Table 3.11a

Association of blood groups with microfilaraemia.

Blood groups	Microfilaraemics			Amicrofilaraemics		
	Female (n = 273)	Male (n = 455)	Total (n = 728)	Female (n = 363)	Male (n = 353)	Total (n = 716)
A	59	84	143	87	87	174
B	88	161	249	106	121	227
O	116	182	298	152	124	276
AB	10	28	38	18	21	39

Comparison between microfilaraemics versus amicrofilaraemics
(i) Female : Chi square value = 1.46; P value = 0.70; (ii) Male : Chi square value = 4.92; P value = 0.18
(iii) Total : Chi square value = 4.81; P value = 0.19

Table 3.11b

Association of blood groups with disease.

Blood groups	Clinically postive Female (n=255)	Male (n=205)	Total (n=460)	Clinically negative Female (n=381)	Male (n=603)	Total (n=984)
A	61	41	102	85	130	215
B	77	79	156	117	203	320
O	100	75	175	168	231	50
AB	17	10	27	11	39	399

Comparison between clinically positive and clinically negative.
(i) Female : Chi square value = 6.01; P value = 0.11; (ii) Male : Chi square value = 1.97; P value = 0.58
(iii) Total : Chi square value = 1.06; P value = 0.79

Table 3.11c

Association of blood groups with microfilaraemia (mF) and disease (Cln) status in combination.

Blood groups	I mF -ve & Cln -ve (n=128)	II mF -ve & Cln +ve (n=600)	III mF +ve & Cln -ve (n=332)	IV mF +ve & Cln +ve (n=384)	III vs IV p Value
A	22(17.2)*	121(20.2)	80(24.4)	94(24.5)	0.221
B	51(39.8)	198(33.0)	105(31.6)	122(31.8)	0.070
O	49(38.3)	249(41.5)	126(38.0)	150(39.1)	0.251
AB	6(4.7)	32(5.3)	21(6.3)	18(4.7)	0.383

* Figures in parentheses show the % of 'n'.

the model and a table of analysis of deviance was constructed to see the significance of the factors influencing disease and infection. It was found that for both infection and disease status, blood group did not contribute significantly as shown in the Table 3.12. But age was found to be significant both for infection and disease status as expected. Infection in the case of disease and disease in the case of infection status were observed to be significant contributing factors as expected.

3.2.4. Distribution of clinical cases and microfilaria carriers in relation to family size in Pondicherry.

Earlier studies carried out at the VCRC had indi-

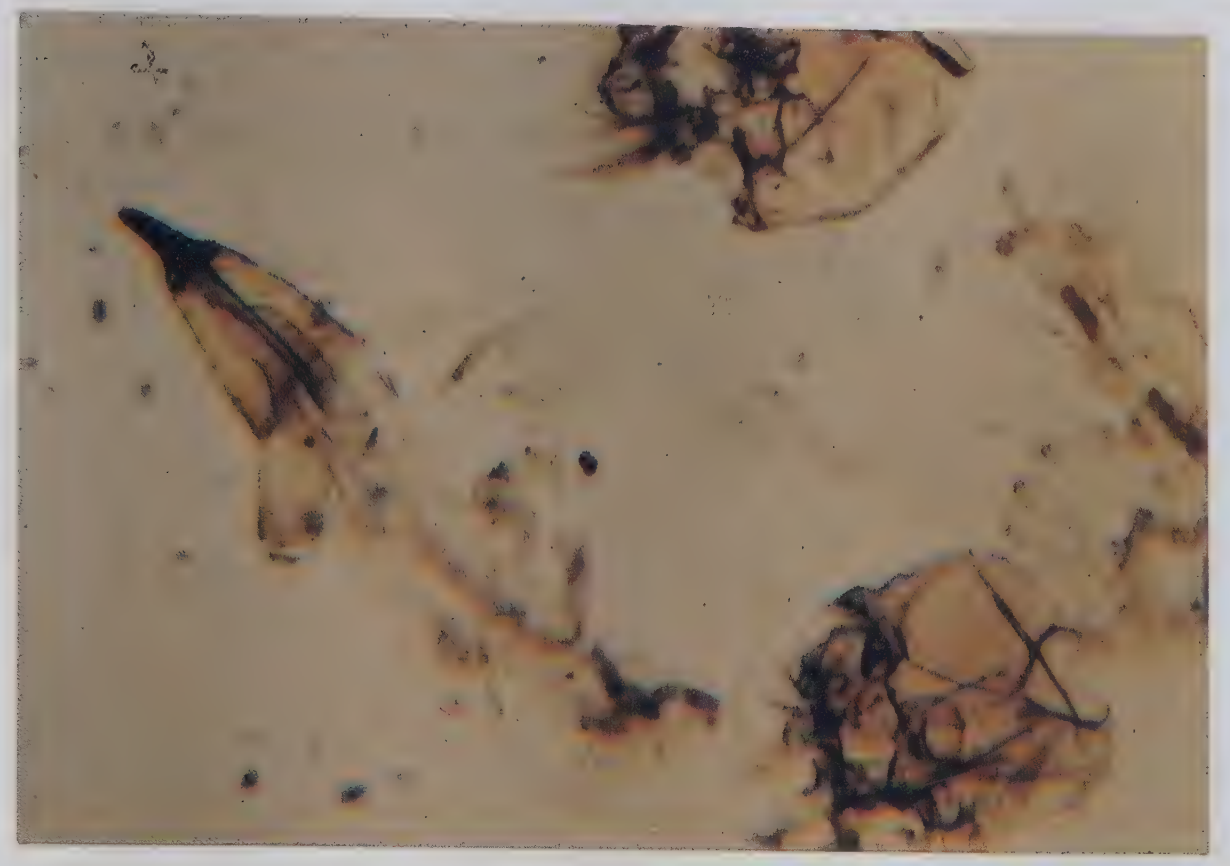
A. Prof. A.S. Paintal, Director General of ICMR, the Guest of Honour in a Health education campaign at Shertallai (Section 1).

B. Dr. S.P.Tripathy, Additional Director General of ICMR, inaugurating a "Filariasis Detection and Treatment Centre" at Shertallai (Section 1).

PLATE II

A. Collection of resting mosquitoes of Mansonioides from outdoor shelters using a "Drop-net cage" (Section 1.1.2).

B. An "Exit Trap" used to study the house frequenting behaviour of Mansonioides mosquitoes (Section 1.1.2).



A. Remnants of Mansonioides larvae in the gut contents of fingerlings of *O. goramy.* (Section 1.2).

B. "Floating cages for studying larvivorous potential of fishes under natural conditions (Section 1.2).

PLATE IV

A. The tribals in Koraput area usually spend the winter evenings outdoors, near the fire (Section 2.2.2).

B. *An fluviatilis* adults being collected from a pit shelter (Section 2.2.2).

PLATE V

A. A Streptomycete colony which yields highly active mosquitocidal metabolite (Section 4.2).

B. Spray-drying equipment used in the down stream processing of Bacterial larvicides (Section 4.3).

PLATE VI

A. Spraying operations by a woman employee of Cochin Corporation (Section 7.1).

B. Defective septic tanks with the effluents flowing out – a major mosquito breeding source at Cochin (Section 7.1).

A. Candidates seeking admission to M.Sc (Medical Entomology) Course have to appear at All–India Written Entrance test (Section 8.1).

B. M.Sc. Students during a study tour (Section 8.1).

PLATE VIII

A. Prof. W.W.Macdonald, who chaired the Seminar on "Future research needs on Lymphatic Filariasis" (8-10th October, 1990), speaking at the inaugural session (Section IV.3)

B. Proceedings of the Seminar in progress (Section IV.3)

Table 3.12

Analysis of deviance table obtained by fitting a logistic model to identify the importance of blood group, microfilaria count (mFc) and clinical status of an individual.

Factors	Infection Status Deviance	df	p value	Clinical status Deviance	df	p value
Blood groups (a)	3.83	3	0.281#	1.06	3	0.788#
Age (b)	14.30	2	0.001	86.61	2	0.000
Disease (c)	128.10	1	0.001	NA	NA	NA
mFc (d)	NA	NA	NA	141.43	1	0.000
(a) + (b)	18.39	5	0.003	87.45	5	0.000
(a) + (c)	131.61	4	0.000	NA	NA	NA
(a) + (d)	NA	NA	NA	142.74	4	0.000
(a) + (b) + (a)*(b)	18.44	6	0.005	87.84	6	0.000
(a) + (c) + (a)*(c)	131.92	5	0.000	NA	NA	NA
(a) + (d) + (a)*(d)	NA	NA	NA	142.81	5	0.000

\# : The deviance or the sum of squares is insignificant.
df : degrees of freedom; NA : Not Applicable.

cated that mF carriers are clustered in families with more than 5 members in a rural area (VCRC annual report, 1989). However, the distribution of clinical cases among the households in a population was not considered. In the present analysis, the data base from clinical and parasitological surveys carried out in Pondicherry in 1986, was used to examine the distribution of clinical cases as well as mF carriers. These analyses are important to decide the target microfilaria carrier group for delivering chemotherapeutic measures. The distribution analyses was carried out separately for the following three groups: (i) cases with chronic filarial disease manifestations (irrespective of their mF status), (ii) microfilaria carriers (irrespective of their clinical status), and, (iii) all cases either with chronic manifestations or microfilaraemia or with both. The following two methods were used for the analyses. (A) The number of persons surveyed in a family was considered as the family size irrespective of the actual size of the family, and, (B) considering the actual family size (those households where coverage was complete in the survey) following earlier method (VCRC annual report, 1989). As the number of infected individuals was low in family sizes 3 and below, these households were considered as one group. Thus the households were classified into three groups viz. (i) $< = 3$ (ii) 4 & 5 and (iii) above 5 individuals. Two statistical approaches were made to study the actual distribution of cases (i) Poisson distribution, to see the randomness and (ii) Negative binomial distribution, to see the aggregation or clustering pattern of cases. The expected frequency distribution of numbers of infected individuals per household was estimated using the Poisson distribution,

$$P(x) = e^{-k}k^{x}/x! \quad x = 0,1,2.....$$

Where: 'P(x)' is the probability of having (x) infected individuals per household; and 'k' is the mean of the Poisson distribution. The observed and expected frequencies were then compared for different groups using a Chi-square goodness of fit test. When the data did not fit Poisson distribution, negative binomial distribution which represents clumping distributions was chosen for testing the non-randomness of infected individuals. The expected frequency distribution of number of infected individuals per household was thus estimated using the method of maximum likelihood estimation for negative binomial distribution

$$P(x=k) = \binom{a+k-1}{a-1} * (b/(b+1))^a (1/(b+1))^k$$

Where 'a' and 'b' are parameters to be estimated. The observed and expected frequencies were then compared using a Chi-square test for goodness of fit.

A total of 1,170 households was visited for clinical examination. The distribution of all households visited, in relation to actual family size (Fig. 3.18) showed that the average number of individuals per household was 5.

A. Analysis of the distribution of filariasis cases in the households where the number of participated individuals were taken as family size.

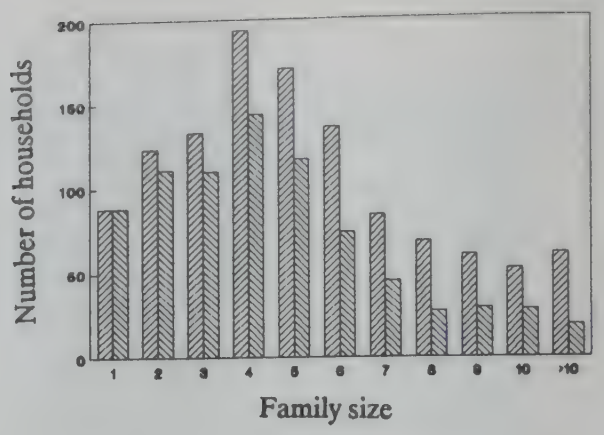


Fig. 3.18 **Distribution of households that were enumerated (▨) and of those households with full coverage (▨) in relation to family size.**

(i) Clinical cases with chronic manifestations:

All 1,170 households visited and covered under clinical survey were classified according to three classes of family size (i.e. < =3, 4 & 5, and, above 5). Expected frequencies of households that had varying number of clinical cases were computed based on Poisson distribution, for each class of family size. For all the classes, the Poisson distribution provided a good description of the observed trend (Table 3.13),

Table 3.13

Comparison between observed numbers of households containing varying numbers of filarial diseased persons and expected frequency based on Poisson distribution.

	Group I		Group II							
			< =3		4 to 5		>5		Total	
No.diseased	O	E	O	E	O	E	O	E	O	E
0	599	595.4	375	375.1	268	271.1	219	218.9	862	859.4
1	165	170.0	64	64.1	100	93.0	99	98.3	263	265.2
2	23	24.2	6	5.5	11	15.9	20	22.1	37	40.9
3	5	2.3			3	1.8	5	3.3	8	4.2
Chi square		0.2479		0.0513		1.3406		0.0114		0.0258
P value		0.6185		0.8210		0.2469		0.9151		0.8724

Group - I : No. of households with full participation (Total)
Group - II : No. participated was taken as family size
O - Observed; E - Expected.

indicating that the cases are randomly distributed. The distribution of cases with hydrocele and elephantiasis was also found to be random as described by Poisson probability model (χ^2 = 0.03; P = 0.87).

(ii) Parasite carriers:

In the clinical survey sites, a total of 1,035 households were covered during the microfilaria survey. In the households of family size above 5, the distribution of mF carriers did not fit the Poisson probability statistics (χ^2 = 23.18; P < 0.001), but it was described by negative binomial model (Fig. 3.19), which gave a good description of the trend (χ^2 = 4.43; P = 0.11) indicating that mF carriers are clustered when size of the family was more than 5 individuals. Though the proportion of households which have a family size of more than 5 individuals in the population was 39%, these contributed 62% of mF carriers.

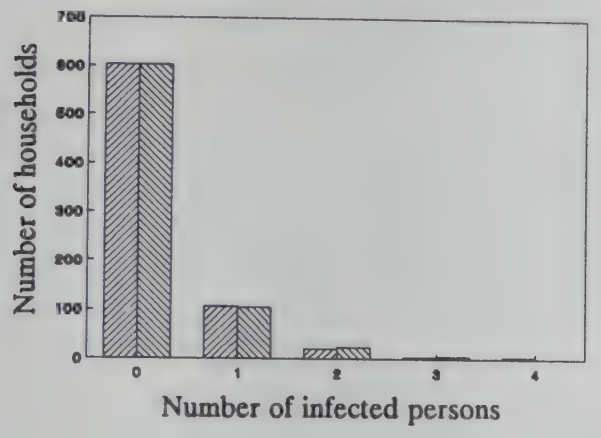


Fig. 3.19 Distribution of households observed (▨) and expected (▧) in relation to number of microfilaraemia cases.

(iii) Filariasis cases either microfilaraemic or with chronic manifestations:

A total of 2,339 persons was examined both for microfilaria and clinical manifestations and they were distributed over 737 households. To determine the distribution pattern of filariasis cases (either microfilaraemic or with chronic manifestations or both) among these 737 households, Poisson distribution was fitted for each class of family size. For the two classes of family size viz. 1 to 3 and 4 to 5, Poisson distribution was found describe the data adequately. The expected and observed frequencies for these two classes and the probability level for goodness of fit are presented in Table 3.14.

As the Poisson distribution did not describe the trend in households with family size of more than 5 and also for total (without considering the family size), the ex

Table 3.14

Comparison between observed numbers of households containing varying numbers of infected persons with *W. bancrofti* and expected frequency based on Poisson and negative binomial distributions.

Infected	Group I				Group II			
	< =3		4 to 5		>5		Total	
	O	E	O	E	O	E	O	E
0	356	359	96	94	32	34	484	486
1	105	99	58	61	35	30	198	191
2	11	14	19	20	11	14	41	48
3	1	1	7	4	3	5	11	10
4					3	1	3	2
Chi square		0.9549		0.3330		1.5530		1.8080
P value		0.3285		0.5639		0.2127		0.1788

Group I : Poisson distribution for Family size; Group II : Negative binomial distribution for Family size.
O : Observed; E : Expected.

pected frequencies were computed using negative binomial distribution which adequately described the distribution pattern (Table 3.14).

Since the average number of filarial cases in the households with size of more than 5 was double the average number of diseased persons, this itself could influence the distribution of cases. The gender distribution in both the groups of households (family size 5 or less than 5 and the family size more than 5 individuals) was similar ($\chi^2 = 0.43$; $P = 0.51$). However, the age structure of population sampled in these groups was found to be heterogeneous ($\chi^2 = 14.09$; $P = 0.02$). In the households with family size above 5, out of those sampled, the proportion of below 20 years of age (0.59) was significantly higher ($Z = 3.41$; $P < 0.05$) than that of the households with 5 or less than 5 individuals (0.51). However, the proportion of mF positives (33% and 35% for family sizes $<= 5$ and above 5 respectively) in this age class was not significantly different ($P > 0.05$) between the two groups of family sizes. The overall filariasis rate (persons either microfilaraemic or with chronic manifestations or both) was found to be 14.41% and 12.48% in the households with family size of 5 and below, and, above 5 respectively. There was no significant ($\chi^2 = 1.27$; $P = 0.26$) difference between the two groups. There was a statistically significant association between filariasis and age of the individuals in both the groups of family sizes (Chi-square test for linear trend in proportion: for family size $<= 5$, $\chi^2 = 53.34$; $P = 0.00$ and for family size above 5, $\chi^2 = 30.19$; $P = 0.00$) indicating that the total filariasis rate was age dependent (Fig. 3.20).

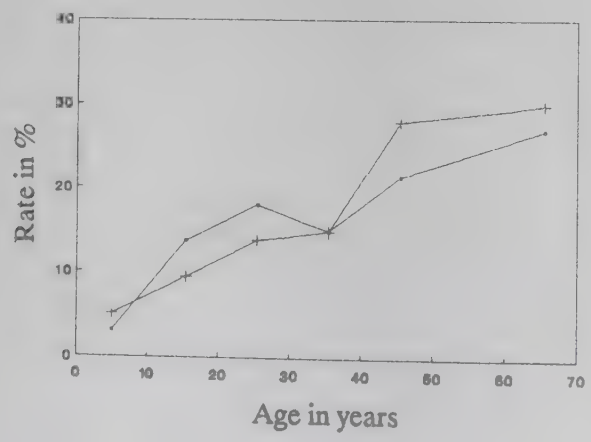


Fig. 3.20 Agewise filarial infection rate in two classes of family size: household size ≤ 5 (•) and household size > 5 (+).

B. *Analysis of the distribution of clinical cases in the house holds where all members participated.*

Out of 1,170 households visited, all the family members were covered only in 792 households (since the occupants in the other households had left the area for occupation elsewhere). When the family size increased, the proportion of households with full coverage decreased (Figure 3.18). However, a minimum coverage of 40% of the households was achieved in different family sizes. The average number of individuals per household with full coverage was 4 which was not significantly different from that of all enumerated households (5).

(i) Cases with only clinical manifestations:

Classification of these 792 households was done according to varying numbers (0-3) of clinical cases per household. Poisson distribution was fitted to this data and it was found to be a good fit suggesting that the distribution pattern is random (Table 3.13).

These analyses showed that while the distribution of mF carriers was clustered, the distribution of clinical cases was random in Pondicherry. The distribution of clinical cases did not seem to be influenced by the distribution of mF carriers, which was clustered. There may be several factors influencing such randomness of clinical cases. Disease prevalence is more in the age groups of above 20 years and the population in this age group is distributed randomly. It appears that difference in distribution of young and old age classes in different family sizes simply but crucially decides the patterns of distribution of mF carriers and diseased individuals.

3.3. CHEMOTHERAPY:

3.3.1. Clearance of microfilaraemia following Diethylcarbamazine (DEC) therapy: relation with age, sex, microfilaria count and clinical status.

Chemotherapeutic measures have become very important, since the benefits of vector control alone (even when done adequately) does not accrue rapidly (Final report of Filariasis control demonstration project, VCRC, 1987). This is due to the prolonged fecundic life span of adult female parasite and the complex population dynamics of the parasite. For rapid and sustained reduction in reservoir of infec-

tion, therefore, chemotherapy should be undertaken concurrently. This in turn necessitates identification of factors that decide the degree of microfilaria clearance following DEC therapy. For this purpose, the large data set available at the VCRC filariasis clinic was analysed.

Microfilaria carriers who underwent DEC therapy at the Filariasis clinic, were followed for parasite clearance. Each course consisted of 6 mg of DEC per kilogram of body weight in three divided doses daily for 12 days as per the WHO recommendations. The regularity of tablet intake was checked by subsequent house visits and tablet counting. Those who had discontinued the tablets or had taken the tablets irregularly were excluded for further follow up. All mF carriers were asked to visit the night clinic for parasitological follow-up at least 4 times after completion of 1st course of DEC (within 30, between 31-90, between 91-180, and, between 181 - 360 days after the course of DEC). Whenever mF reappeared, the carriers were again given another DEC course (same dosage as 1st course).

A total of 1,428 mF carriers attended the filariasis clinic and received DEC. Only 729 mF carriers (305 females and 424 males) who took the Ist course DEC tablets regularly were followed for night blood parasitaemia. The age of these mF carriers ranged between 4 and 75 years (the mean age in males was 25.67 $\pm$ 0.60 years and in females it was 25.38 $\pm$ 0.78 years). The mean pre-therapy mFc for the 729 carriers was 11.5 $\pm$ 0.9, and, it was 12.3 $\pm$ 1.4 in males and 10.3 $\pm$ 0.9 in females (difference between the sexes: t = 1.1; P = 0.30). The mean period of follow up was 288.1 $\pm$ 7.4 days. The coverage of mF carriers at different time intervals after completion of 1st course DEC is shown in Table:3.15.

The coverage ranged between 51.3% to 68.3% and it could not be higher due to practical constraints, in spite of the motivation of the mF carriers to attend the clinic regularly. Only 105 mF carriers were followed regularly all the four times. However, there was no significant difference in mF positivity rate after 1st DEC course among those followed up regularly (40/105: 38.1%) and others (238/624: 38.1%) (between the groups: χ^2 = 0.01; P = 0.92). Also, the mean duration of becoming mF positive following 1st DEC course for both the groups (85.3 $\pm$ 11.1 days for the 40 cases out of 105 and 92.4 $\pm$ 6.4 days in 238 cases out of 624 cases) did not vary significantly (t = 0.44; P = 0.66).

Table 3.15

Coverage of followup of mF carriers at different time intervals.

Mean duration* of follow up $\pm$ S.E (in days)	Number of cases followed up	% of cases followed up (% of n=729)	% mF +ve of the cases examined
24.9 $\pm$ 0.2	374	51.3	18.7
63.7 $\pm$ 0.7	495	67.9	23.2
142.4 $\pm$ 1.1	498	68.3	16.5
301.5 $\pm$ 9.0	384	52.7	14.6

* : Duration after receiving Ist course of DEC.

Factors influencing microfilaraemia (mF) clearance by DEC:

(i) Number of DEC courses: The results of mF positivity following different courses of DEC are shown in Table 3.16.

The proportion of mF carriers who turned mF negative increased from 61.9% after the 1st course to 100% after the 4th course. The mean mFc before and after the corresponding DEC course in mF carriers who received DEC course is shown in Table 3.17. A significant reduction in mean mFc was observed following each course.

(ii) Mean microfilaria count: Comparison of pre-therapy mean mFc between cases who became mF negative and those who became mF positive after each course of DEC is shown in Table: 3.18.

Irrespective of the course of DEC, the mean mFc prior to the corresponding course was significantly higher in cases who subsequently became mF positive compared to those who became mF negative. The mean pretreatment mFc of cases which required only 1 course DEC was the lowest and those requiring 4 courses was the highest (Table 3.19).

Table 3.16

Details of clearance of mF in relation to DEC courses.

No. of DEC Course	mFcarriers received DEC (n)	mF negative		mF positive	
		% of 'n'	Mean duration# of follow up ± S.E. (in days)	% of 'n'	Mean duration# of becoming mF +ve ± S.E.(in days)
1st course	729	61.9	204.5 ± 8.3	38.1	97.4 ± 6.9
2nd course	183	73.2	175.9 ± 17.0	26.8	79.2 ± 12.8
3rd course	34	85.3	106.4 ± 16.1	14.7	64.2 ± 17.0
4th course	5	100.0	33.0 ± 6.4	0.0	NA*

* : NA - Not Applicable
\# : Duration after the corresponding DEC course.

Table 3.17

Comparison of pre and post mean mFc in cases who received different rounds of DEC therapy.

DEC course	Number received DEC (n)	Mean microfilaria count*		Significance between the means	
		Pre course	Post course	't' val.	p Value
1st	729	11.5 ± 0.9	1.1 ± 0.1	12.0	0.0
2nd	183	3.0 ± 0.4	0.5 ± 0.1	-5.8	0.0
3rd	34	1.7 ± 0.2	0.3 ± 0.1	9.1	0.0
4th	5	1.8 ± 0.6	0.0	-	-

* : Mean mFc of 'n' in relation to corresponding DEC course.

The mean mFc following each course of DEC was higher in cases which required higher number of courses. The proportion of cases who became mF positive after 1st course of DEC increased with the rise in the initial mFc: 23.6% in cases with mFc 1 to 100% in cases with mFc more than 100 (Fig. 3.21; association between the two factors: r = 0.97 and P = 0.001). Further, it was also observed that the pre-therapy mean mFc also influenced the mean duration, after which cases became mf positive (after the

Table 3.18

Comparison of mean microfilarial count of cases who became mF -ve with those who became mF +ve following different courses of DEC therapy.

DEC Course	Number became mF -ve (n1)	Mean mFc* of n1	Number became mF +ve (n2)	Mean mFc* of n2	Significance between means n1 vs n2 p value
1st	451	7.3 ± 0.5	278	18.2 ± 2.1	0.000**
2nd	134	2.3 ± 0.3	49	4.9 ± 1.3	0.007**
3rd	29	1.5 ± 0.1	5	3.0 ± 0.9	0.004**
4th	5	1.8 ± 0.6	-	-	-

* : Mean mFc prior to the corresponding DEC course.
** : Significantly different

Table 3.19

Comparison of mean microfilarial count in cases who required different courses of DEC therapy for clearance of mF.

No. of DEC courses	1 course	2 courses	3 courses	4 courses	Significance between the means p value		
No. of cases turned mF -ve	451	134	29	5	1 vs 2	2 vs 3	3 vs 4
	Mean mF count ± S.E.						
Prior DEC (Initial)	7.3 ± 0.5	11.7 ± 1.3	42.0 ± 13.7	42.2 ± 19.6	0.000**	0.000**	0.995
After Ist course	0.0	2.3 ± 0.3	3.7 ± 0.9	15.8 ± 11.2	-	0.079	0.016**
After 2nd course	NA*	0.0	1.5 ± 0.1	3.0 ± 0.9	-	-	0.004**
After 3rd course	NA	NA	0.0	1.8 ± 0.6	-	-	-
After 4th course	NA	NA	NA	0.0	-	-	-

* NA : Not Applicable; ** Significantly different

1st DEC course): in cases with mFc 1, the mean duration was 114.8 days and for cases with mFc greater than 100, this duration was 50.2 days (Fig. 3.22; association between the two factors: r = 0.92 and P = 0.009).

(iii) Age and gender : The overall and age specific mF positivity following the 1st DEC course was not significantly different between sexes (Table 3.20).

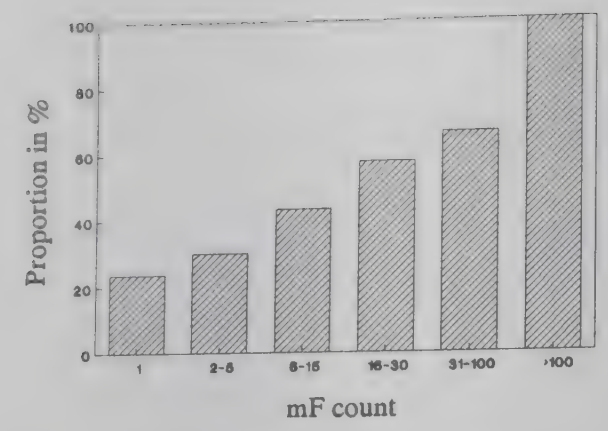


Fig. 3.21 Relationship of proportion of cases becoming mF + ve after first course of DEC and their pretherapy microfilarial counts (20 Cubic mm).

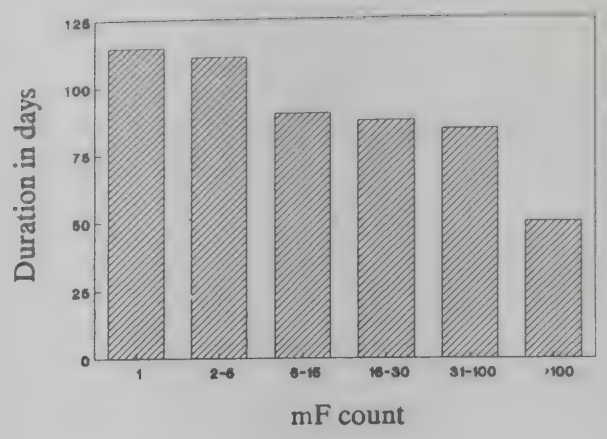


Fig. 3.22 Relationship of pretherapy microfilaria count (20 Cubic mm.) and duration after which the cases became mF + ve, following first DEC course.

The results after second course of DEC were also similar (P > 0.05 between the sexes for all age classes). In both sexes, the reduction of mean mFc from pre- therapy level (males: 12.3 $\pm$ 1.4; females: 10.3 $\pm$ 0.9) to post 1st DEC course (males: 1.2 $\pm$ 0.2; females: 0.9 $\pm$ 0.1) was significant (P = 0.000). It was also noted that there was no association between the age of the mF carriers and their event of becoming mF positive after 1st course of DEC (r = 0.1; P = 0.9, see Table:3.20). Similar results were also obtained after the 2nd course of DEC (r = 0.3; P = 0.6).

(iv) Clinical status: Of the 729 mF carriers 98 had clinical manifestations of filariasis. There was no significant difference in the pre-treatment mean mFc between the symptomatic (14.1 $\pm$ 4.1) and the asymptomatic (11.0 $\pm$ 0.8) individuals (t = -1.2; P = 0.2 between the groups). The proportion positivity in the two groups (35.7% in symptomatic and 38.5% in asymptomatic persons) was also not significantly different (χ^2 = 0.18, P = 0.7).

The results of the present study suggest that; (a) Single course of DEC as recommended by WHO, failed to clear microfilaraemia in as many as 38.1% of carriers though considerable reduction in the parasite density was observed. (b) The clearance of

Table 3.20

Comparison of age and gender specific mF positivity rate after Ist course of DEC.

Age class in years	Males		Females		Significance of proportion male vs female	
	Number examined (n1)	% of 'n1' who became mF + ve	Number examined (n2)	% of 'n2' who became mF + ve	Chi-square	p-value
0-10	18	44.44	22	31.82	0.24	0.62
11-20	157	36.94	123	35.77	0.01	0.94
21-30	133	41.48	70	35.71	0.42	0.52
31-40	59	44.07	45	37.78	0.20	0.66
>40	55	32.73	45	42.22	0.59	0.44
Total	424	39.15	305	36.72	0.35	0.55

the microparasite was related to the pre-treatment mFc. (c) Longitudinal follow up of mF carriers showed that there is a decreasing trend in the mean mFc following each course of DEC. The results of present study call for rationalizing the dosage schedule and it appears that the quantity of the drug to be given should ideally be decided on the pre-treatment mF count. But one has to recognize the difficulties in deciding the end point of DEC therapy. Whether the disappearance of microfilaria is a result of a temporary phenomenon or due to permanent sterilization of adult female parasite or due to its death, is difficult to know.

3.3.2. Long term clinical and parasitological consequences of DEC therapy:

There has been considerable speculation about the long term clinical and parasitological consequences of DEC therapy. The Pondicherry data set provided a unique opportunity to examine this. Microfilaria carriers detected in the parasitological survey (1981) had been referred to the local filariasis clinic for treatment. However, only a small proportion of these mF carriers received one course of DEC. Though not intended, this gave an opportunity to examine the clinical and parasitological consequences in relation to DEC therapy over the 5 year period.

A total of 293 mF carriers of 1981 (asymptomatic in 1981) were examined at the VCRC filariasis clinic in 1986. Treatment details for 1981 were available only for 286 individuals. Of these, (a) 207 (73.14%) had not received DEC, (b) 61 persons had received a single course of DEC and (c) 18 persons had discontinued DEC due to side reactions of the drug. After the 5 year period, 39.6%, 72% and 50% of the above three groups respectively had become mF negative (comparison of proportion loss between groups a and b: $\chi^2 = 18.7$, $P = 0.00$; between groups a and c: $\chi^2 = 0.37$, $P = 0.54$). The mean mF intensity had marginally increased in untreated persons (9.4 in 1981 and 10.7 in 1986: $P = 0.41$), but it had declined in those who completed DEC course (14.0 in 1981 to 9.5 in 1986: $P = 0.23$) and those who were partially treated (13.2 in 1981 and 7.4 in 1986: $P = 0.098$). Clinical examination revealed that 22 (10.6%), 9 (14.8%) and 4 (22.2%) in groups a, b and c had developed filarial disease in 1986. The acute and chronic disease rates in three groups were 4.3%, 4.9%, 5.5% and 5.8%, 9.8%, 16.7% respectively. Statistical analyses however, did not reveal any significant difference either in acute or chronic disease rate between group a and others.

The analyses showed that DEC results in reduction in intensity of microfilaraemia (though the dosage is not sufficient for complete clearance as shown vide 3.2.1.). The risk of developing chronic disease appears to be higher in those who received DEC (particularly those who developed side reactions and discontinued, though statistical analyses did not show any significant difference). Further simultaneous clinical, parasitological and immunological investigations will be necessary to understand the interaction of different factors on the time scale.

3.3.3. Impact of mass biannual single dose DEC therapy on prevalence and transmission:

This study was undertaken (between January, 1988 and January, 1990) in Kottakuppam, a semiurban area, adjacent to Pondicherry, which recorded microfilaraemia prevalence of 6.7% in 1986. The total population covered was 2,233 in 317 households with 396 families. An attempt was made to give DEC at the dosage of 6mg/kg in a single dose twice a year for two years to the entire population. The impact of DEC therapy was monitored by repeated random blood surveys and fortnightly monitoring of the vector population.

The coverage of DEC was above 80% of target population (people were made to consume the DEC in the presence of the staff) in all 4 rounds. The precontrol microfilaraemia prevalence and its mean intensity (20 cubic mm) were 6.0% and 0.7 respectively. These were reduced significantly ($P < 0.05$) to 2.1% and 0.17 respectively after the 4 rounds of DEC (Table: 3.21).

Analyses of data on parasite population in vector showed that both infection and infectivity rates showed a declining trend. The average number of mF and infective stage (L_3) showed this trend particularly clearly (Table 3.22). The results indicate that biannual single dose DEC results in a declining parasite load in both human and vector population.

3.4 ENTOMOLOGICAL ASPECTS

3.4.1. Biting periodicity index of Culex quinquefasciatus:

Table 3.21

Coverage of population for DEC therapy and its impact on microfilaraemia prevalance and intensity.

Prior/ after	DEC course	DEC coverage of population (%)	Population covered in blood survey (%)	Mf rate (%)	Mean intensity
Prior	I	89.2	55.9	6.0	0.7
Prior	II	82.7	26.4	6.3	0.5
Prior	III	82.5	21.3	5.0	0.3
Prior	IV	83.6	22.6	4.1	0.7
After	IV	-	30.6	2.1	0.2

Table 3.22

Impact of DEC therapy on vector parameters.

Prior/ after	DEC course	Man hour density	Prop. infec ted	Prop. infec- tive	Per infective mosquito		
					mf	L3	any stage
Prior	I	21.54	9.86	0.95	9.40	1.35	7.69
Prior	II	29.03	8.99	1.09	9.09	2.89	7.75
Prior	III	38.08	8.40	0.54	8.78	1.90	5.68
Prior	IV	36.83	7.97	0.86	4.34	1.00	5.58
After	IV	17.08	7.33	0.17	3.13	0.50	5.17

The biting periodicity index of the bancroftian filariasis vector, *Cx. quinquefasciatus*, was calculated using hourly data obtained from all night biting (18.00-06.00 hrs) collections. The biting rhythm of *Cx. quinquefasciatus* is assumed to be of "Harmonic wave type". The relationship between mosquito biting density (Y) and hour of night (h) was studied by modification of an earlier method.

The least square estimations of mean (m) and constants (b and c) of multiple regression equation were computed by the modified method. Hours have been chosen in such a way that satisfies the condition cos 30 h = sin 30 h = 0 and the parameters m, b and c have been estimated using the number of mosquitoes collected during the individual hours of the night.

The periodicity index, which is the coefficient of the variation of no. of mosquitoes collected during certain time intervals over a period of 12 hours, was calculated by dividing the standard deviation of man hour density with mean density.

The biting periodicity index of *Cx. quinquefasciatus* was 29.78, when a duration of 12 hours was taken into account, and found to be close to the periodicity index of the microfilariae of *W. bancrofti*. The peak biting activity of the vector and peak appearance of mf in the peripheral blood occurs at about 01.00 hrs and this enables the optimum infection of the vector population.

3.4.2. Seasonal variation in the transmission of bancroftian filariasis in Pondicherry:

Various studies in different parts of the world suggest that seasonal variations in the density of *Cx. quin-*

quefasciatus occur in some places. However, information on the transmission pattern of bancroftian filariasis is scanty. Monthly analysis of data from biting collections indicated that the host seeking density and parous biting density of *Cx. quinquefasciatus* varied and they tended to be lowest in the summer months of May and June and also in the month of July. The greatest density was observed in the month of January. The seasonal pattern was well pronounced for infective mosquitoes also. No infective stage larvae were found in the mosquitoes dissected during the period that comprises peak summer month of June and early monsoon months of July and August. The frequency of infective biting mosquitoes was highest in the month of November and January (Fig. 3.23).

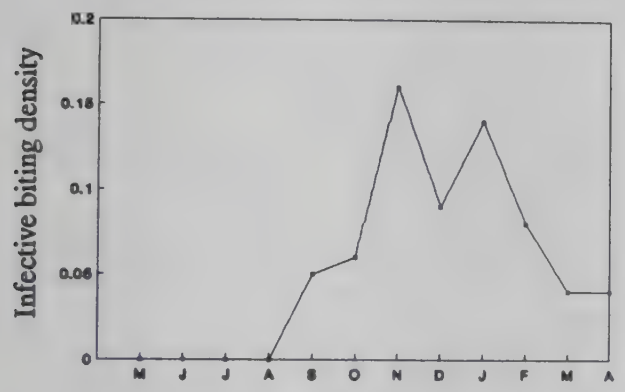


Fig. 3.23 Infective biting density of *Culex quinquefasciatus* during different months.

The quantity of transmission, expressed in terms of Monthly transmission potential, was also maximum in the month of January and then declined gradually until April and was 0 from May to August. Substantial transmission started again in September and continued till December with a high level in November. Transmission in the months of November and January accounted for 34.7% and 21.9% respectively of the Annual transmission potential, which is the sum of the 12 monthly transmission potentials in a year. About 80% of the transmission occurred between November and March. The results of the study indicate that intensification of control methods during the peak transmission season may produce better results than a year round control programme.

3.4.3. Spatial and temporal changes in resting density, filarial infection and infectivity rates of *Cx. quinquefasciatus:*

The evaluation of population of *Cx. quinquefasciatus* is being continued in Pondicherry. Longitudinal data on fortnightly/monthly changes and place dependent variations in important entomological parameters such as resting density and filarial infection and infectivity rates of this important vector population are available with VCRC. The data consist of 2 sets, one belonging to that collected when there was no control pressure and the other when the vector population was under control pressure. These data were subjected to statistical test using GLIM programme.

The vector density differed significantly between 17 sites during pre-control ($F = 16.95$; $P < 0.005$) as well as control ($F = 21.24$; $P < 0.005$) periods suggesting that the mosquitogenic potential of the sites varied. The fortnightly variations in the average resting density of 17 sites were highly significant during pre-control ($F = 2.73$; $P < 0.005$) and control ($F = 3.216$; $P < 0.001$) periods. The seasonal pattern in the density is similar with or without control measures. The control operations substantially reduced the vector population size ($F = 12.28$; $P < 0.0005$). The infection rate, like density, differed significantly (Pre-control - $\chi^2 = 189.9$, $P < 0.0001$; Control - $\chi^2 = 206$, $P < 0.0001$) between sites, suggesting that the prevalence of microfilaraemia and intensity of transmission is not homogenous throughout the area. Infection rate varied significantly between fortnights also.

The changes in infection rate from pre-control to control periods was analysed using a logistic regression model. There was a significant reduction in the infection rate of 14 IVM sites from pre-control to the control period ($\chi^2 = 13.87$, $P < 0.05$). The overall risk of infection of the vector population was reduced from 100% in the pre-control period to 83.37% during the control period. Without the IVM programme, the risk of infection of the vector population would have increased by a factor of about 1.2.

Comparison of vector infection rate with microfilaraemia prevalence in humans of 17 sites, using log linear models, indicated that there was a close association between the two parameters (Pre-control - $\chi^2 = 9.126$, $P < 0.05$; Control - $\chi^2 = 6.74$, $P < 0.05$).

The infectivity rate also showed significant site-wise (Pre-control - $\chi^2 = 22.32$, $P < 0.001$; Control - $\chi^2 = 26.96$, $P < 0.005$) and fortnightly variations (Pre-control - $\chi^2 = 40.37$, $P < 0.05$; Control - $\chi^2 = 39.83$, $P < 0.05$). However, it did not show any association with mf prevalence in humans. The mean L3/infective mosquito showed significant variations between the sites and no significant fortnightly variations were observed.

The analysis showed that the intensity of transmission differs even in a contiguous area like Pondicherry. Earlier studies indicated that the microfilaria carriers also tend to cluster. So, within a town or city high risk areas should be identified and given the due attention to tackle the problem more effectively.

3.4.4. Incrimination of vector of rural filariasis:

More than two-thirds of the people affected by lymphatic filariasis live in rural areas in India. However, information on the role played by different mosquito species in the transmission of filariasis is scanty. Therefore, a study was undertaken to elucidate the role of different species of mosquitoes in the transmission of filariasis. Fortnightly entomological surveys were carried out in 5 villages near Pondicherry and the results of these surveys are given in Table 3.23.

Table 3.23

Filarial infection and infectivity rates of different species of mosquitoes in the study villages.

Species	Nd	NP	IR	IV
Kuilapalayam				
Cx.quinquefasciatus	190	13	6.8	0.0
Cx.vishnui gr.	57	1	1.8	0.0
Cx.gelidus	4	0	0.0	0.0
An.subpictus	147	1	0.7	0.0
Ae.aegypti	44	0	0.0	0.0
Alankuppam				
Cx.quinquefasciatus	446	35	7.9	0.9
Cx.vishnui gr.	116	0	0.0	0.0
Cx.gelidus	0	0	0.0	0.0
An.subpictus	490	0	0.0	0.0
Ae.aegypti	1	0	0.0	0.0
Kottakkarai				
Cx.quinquefasciatus	74	6	8.1	0.0
Cx.vishnui gr.	76	0	0.0	0.0
Cx.gelidus	1	0	0.0	0.0
An.subpictus	458	0	0.0	0.0
Ae.aegypti	0	0	0.0	0.0
E.Chavadi				
Cx.quinquefasciatus	237	24	10.1	0.4
Cx.vishnui gr.	49	1	2.0	0.0
Cx.gelidus	0	0	0.0	0.0
An.subpictus	275	0	0.0	0.0
Ae.aegypti	108	1	0.9	0.0
C.M.Chavadi				
Cx.quinquefasciatus	120	1	0.8	0.0
Cx.vishnui gr.	35	0	0.0	0.0
Cx.gelidus	0	0	0.0	0.0
An.subpictus	194	0	0.0	0.0
Ae.aegypti	42	0	0.0	0.0

ND - No.dissected, NP - No.positive for infection, IR - Infection rate, IV - Infectivity rate

An. subpictus was the dominant species followed by *Cx. quinquefasciatus* in these villages. Laboratory dissections were carried out on 5 species viz., *Cx. quinquefasciatus, Cx. vishnui gr., Cx. gelidus, Ae. aegypti* and *An. subpictus*. The proportion of mosquitoes infected in the latter 4 speciess is either zero or very low and no infective stage larva was found in the dissected mosquitoes.

But, considerable proportion of resting population of *Cx. quinquefasciatus* was found infected and this species appears to be the only vector as per the results obtained so far. The indoor resting density of *Cx. quinquefasciatus* ranged from 3.36 in the village Kottakarai to 17.71 in Alankuppam. The highest infection rate of 10.13% was observed in Edayanchavadi and the lowest of 0.83 in Chinnamudaliarchavadi. The infectivity rate varied between 0 and 0.90 in different villages. The entomological surveys are being still continued to study the dynamics of transmission of filariasis in rural areas.

4. BIOLOGICAL CONTROL

During the previous year several new isolates of bacteria, fungi and actinomycetes were obtained from samples collected from different regions of India. Some of these were found to be larvicidal on preliminary screening. Several basic studies on the biology of *Lagenidium* sp. and *Romanomermis iyengari*, and secondary metabolites of *Tolypocladium* sp. and *Bacillus thuringiensis* H-14, were carried out. Deltafix, a slow release formulation of *B. thuringiensis* H-14 was produced and tested in the field. Toxicological studies on *B. sphaericus* were also initiated. During the reporting year while the search for new strains of biocontrol agents and the basic studies were continued further, emphasis was also laid on the production and evaluation of biocides and their by-products viz., Cyclosporine A and L-dopa.

4.1. SEARCH FOR INDIGENOUS PATHOGENS OF MOSQUITOES

Identification and characterization

The serological identification of thirteen *B. thuringiensis* and forty-three *B. sphaericus* isolates (reported in the 1989 Annual report) was carried out. Of the thirteen *B. thuringiensis* isolates, nine belonged to serotype H-14 (*B. thuringiensis israelensis*), one each to the serotypes H-10 (*B. thuringiensis darmstadiensis*) and H-17 (*B. thuringiensis tohokuensis*), one could not be tested due to non-motility of its cells and one did not agglutinate with the antisera of 22 serotypes available with us. Of the forty-three *B. sphaericus* isolates,twenty-one belonged to the serotype H-5a5b, two could not be tested due to non-motility of the cells while twenty were untypeable with the 5 antisera available with us.

Dynamics of growth, sporulation and toxin synthesis in B. sphaericus H-5a5b strains

Significant differences in the pattern of growth, biomass production, sporulation and toxin synthesis were observed among the six *B. sphaericus* H-5a5b strains studied. These were, i) B 42 and B 43 completed the growth cycle in a shorter period and produced higher number of spores, ii) B 42 and B 64 yielded higher quantity of biomass and their spores exhibited higher toxicity, iii) B 85 was a slow grower, yielded lesser biomass and its spores exhibited delayed toxicity, iv) B 45 and B 57 were slow growers, yielded little biomass and spores and they have taken longer time to complete sporulation as well as toxin synthesis.

Shelf-life of B. thuringiensis H-14 and B. sphaericus H-5a5b formulations

Studies indicate that storage of *B. thuringiensis* H-14 and *B. sphaericus* H-5a5b strains as water dispersible formulation (WDP) prevents deterioration of larvicidal activity for a longer time compared to lyophilised cells. Also there was a slight increase in the activity of WDPs during storage. Lyophilised cells had to be hermetically sealed before storage, otherwise there was rapid deterioration. It was also observed that lower the temperature of storage, the longer was the shelf life, of both WDP as well as lyophilized cells. In general, strains of *B. thuringiensis* H-14 had a longer shelf life than strains of *B. sphaericus* H-5a5b, when stored under identical conditions.

Small scale Field evaluation of B. thuringiensis H14 and B. sphaericus H5a5b strains

Two indigenous strains of *B. thuringiensis* H-14, viz., B 300 and B 325 which were shown to have higher toxicity for mosquito larvae than the standard IPS 80 strain in the laboratory tests, were tested in the field against the immatures of *An. subpictus* in the pits in casuarina gardens. The WDP formulation of these strains was applied at the rate of 10X the laboratory Lc50/m^2 of water surface of the habitat. The two isolates, B 300 and B 325, when applied respectively at half and one fourth the dose of IPS 80 produced a similar level of reduction as IPS 80. Similarly, two strains of *B. sphaericus*, viz., B 381 and B 288, which were shown to have higher toxicity for mosquito larvae than standard strain 2362, in the laboratory tests, were also tested in the field in the same way as *B. thuringiensis* strain described above. They brought about the same level of reduction in the immature density of *An. subpictus* as the standard ones, although they were applied at one third to one fourth the lesser dose as compared to the latter.

Protein profile of B. sphaericus strains belonging to different serotypes

B. sphaericus strains belonging to different serotypes were taken up for this study. The proteins were extracted from the lyophilised biomass and analyzed by SDS-PAGE. The major fraction with a MW of 43 kDa was observed in the highly toxic strains viz., 1593, B 42 and B 56. The less toxic strain B 55 also had a faint band at the same position whereas in the non-toxic strain, B 376, this band was absent. Instead it had a band with a MW of 116 kDa. Thus the results show that in all the toxic strains taken up for this study the 43 kDa protein was present while it was absent in non-toxic strains.

Susceptibility of different species of mosquitoes to B. thuringiensis strains

The susceptibility of the six *B. thuringiensis* H-14 strains for III instar larvae of five mosquito species were determined. *Culex tritaeniorhynchus* was found to be the most susceptible, followed by *Cx. quinquefasciatus, Cx. sitiens, Ae. aegypti*, and *An. stephensi*. The Lc50 dose required for the latter four species of mosquitoes was in the range of 1.2 to 2 times, 1.4 to 2.1 times, 6 to 9.4 times and 55.1 to 99.5 times respectively than that required for *Cx. tritaeniorhynchus*.

4.2. MOSQUITOCIDAL METABOLITES FROM INDIGENOUS FUNGI AND ACTINOMYCETES

A screening programme to find out fungi and actinomycetes that produce novel mosquitocidal compounds has yielded a new streptomycete strain. This was isolated from a soil sample collected from paddy field in Pondicherry. The macro- and micromorphological (Plate V, A), cultural, physiological and biochemical properties of this organism demonstrated that it belonged to the *Streptomyces griseus* complex. Bioassays of the crude culture broth produced by this strain had an Lc50 of 1 to 13 ml/ml to mosquito larvae of different species. It was more toxic to *Cx. quinquefasciatus* than to *An. stephensi* or *Ae. aegypti* larvae.

The streptomycete was mass produced using a laboratory fermentor. The active compound was isolated from the culture filtrate and purified. The purified compound was found heat stable and not affected by ultra-violet light (at 254 and 366 nm). It was active against the larvae of all mosquitoes tested, having Lc50 values in the range of 0.4 - 2.7 ml/l. The compound was also active against houseflies and cockroaches. It showed antifungal activity against a wide variety of fungi but lacked antibacterial activity.

It showed no lethal effect on a number of non-target organisms studied, including aquatic insects, copepods, rotifers and fish (*Gambusia affinis*). Acute oral toxicity test conducted on albino mice and rats showed no toxicity.

The efficacy of the insecticide in killing mosquito larvae was determined as in field waters such as paddy fields, casuarina pits and cess pools to find the effect of organic pollution level and microbial activity on the compound. *Cx. quinquefasciatus* larvae were used for the test with cess pit waters and *An. stephensi* larvae were used for the test with paddy field and casuarina pit water. While the Lc50 dosage in paddy field and casuarina pit waters was unchanged, slightly higher dosage (15 - 20%) was required in cess pit waters.

The organism reached stationary phase of growth on the 3rd day of incubation (Fig. 4.1). The production of insecticide started on the 2nd day and was maximum on the 5th day of incubation. The medium containing glucose as carbon source and soybean meal as nitrogen source supported better growth and enhanced the insecticide production. The optimum pH of the medium for cultivation of this organism was 7.1. The insecticide was formed when the pH range was between 7.4 and 8.4. The optimum temperature range for both growth and insecticide production was 28 to 30°C.

4.3. PRODUCTION AND FORMULATION OF LARVICIDAL BACILLI

Production and formulation of Bacillus sphaericus for evaluation

Forty two batches of cellmass were produced in the pilot fermentor and spray dried (Plate V, B). The mean per litre yield of wet and dry cellmass in each batch were 22g and 3g, respectively. The mean Lc50 value of the spray-dried cellmass to *Cx. quinquefasciatus* larvae was 28 ng/ml. This was pulverized and formulated as micro-encapsulted formulation for field evaluation.

Studies on the Fed-batch fermentation of Bacillus thuringiensis H-14

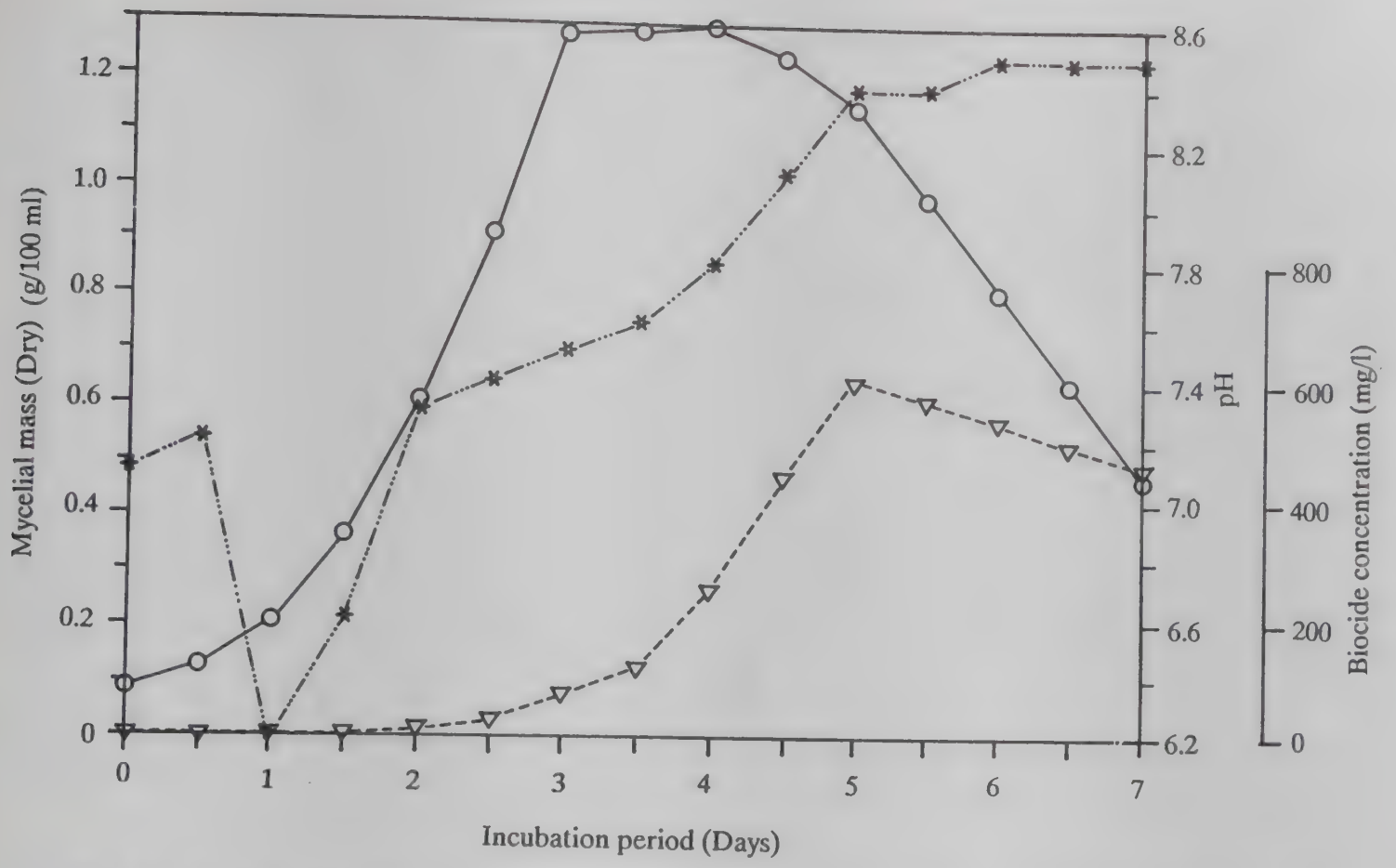


Fig. 4.1 Growth (○) of *Streptomyces griseus* (A81), production of biocide (▽) and change in pH (✱) in soya broth.

Currently, *B. thuringiensis* H-14 is produced by submerged batch fermentation method, in which all the nutrients are added to the medium prior to inoculation and earlier studies indicate the existence of substrate inhibition at higher glucose levels (beyond 2%). Therefore, a study was undertaken to increase the cell density without compromising on the toxin content/cell by adopting a simple fed-batch strategy, with the ultimate objective of producing *B. thuringiensis* H-14 at commercially economical levels.

In two trials, sterile glucose was pumped into the fermentor at the rate of 1.5 g/h/l and 3 g/h/l of culture respectively. Addition of glucose was started after the 3rd h of fermentation and stopped at the end of 16th h of fermentation. Total amount of glucose added during the fed-batch operation was equivalent to 2% and 4% respectively. Normal batch fermentation was carried out with 2% and 4% glucose levels, under identical conditions, for the purpose of comparison.

Batch fermentations of *B. thuringiensis* H-14 using 2% and 4% glucose concentration produced cell mass of 22.5 and 31 g/l, respectively. The Fed-batch approach yielded 43.20 and 80 g/l of cell mass at the substrate feeding rate of 1.5 g and 3.0 g/h/l, respectively. The Lc50 values obtained for the batch cultures grown with 2% and 4% glucose have not shown any difference, inspite of the higher biomass observed at 4% level. Moreover, the level of sporulation observed with 4% glucose was only 60% at the end of 24 h. While Fed-batch culture containing 43.20 g/l of cell mass induced 50% mortality at the dose of 0.12×10^{-2} ml, a dose of 0.05×10^{-2} ml itself was sufficient to induce 50% mortality with culture containing 80 g/l of cell mass. This indicated that toxicity is proportionate to cell yield in the case of Fed-batch fermentation. More than 85% sporulation was observed at the end of 24 h of Fed-batch fermentation.

Results of residual glucose and amino nitrogen at the end of 24 h in batch and Fed-batch cultures showed that higher level of substrate utilization is achieved with the help of fed-batch technique. Sugar utilization of more than 90% and nitrogen utilization of 84% was observed in Fed-batch fermentation using 4% glucose and 1% each of the two nitrogen sources, i.e., peptone and yeast extract (C:N ratio 2:1). On the other hand, glucose and nitrogen were utilized only

to an extent of 50% in the batch mode at the same ratio of carbon to nitrogen.

The data also showed that the level of glucose in the culture medium has never gone beyond 0.4% and was steadily maintained between 0.2 and 0.4% (Fig. 4.2). Toxicity appeared only after 12th h of growth and the culture sporulated uniformly (85%) by the 16th h of growth. Amino nitrogen content of the culture declined sharply during the exponential growth and reached near exhaustion by 16th h of growth during which time the glucose feed also was stopped. However, a slight increase in amino nitrogen was observed after the 20th h. This study established that *B. thuringiensis* H-14 can be grown to high cell densities, without affecting sporulation and toxin expression by adopting this simple Fed-batch model based only on glucose limitation.

Development of a double antibody sandwich ELISA for the quantification of Bacillus sphaericus toxin during fermentation processes

Several studies on the biogenesis of the toxin during different stages of growth of this bacterium have been carried out in the past following different procedures. Though they provide valuable information, the methods adopted have some limitations in that they cannot be put into routine use in the off-line monitoring of *B. sphaericus* fermentations. Therefore an attempt was made to develop an enzyme linked immunosorbent assay (ELISA) for the quantification of *B. sphaericus* toxin.

The *B. sphaericus* strain, B42 was used in this study. The spore coat proteins were isolated from the spores. The major protein showing larvicidal activity, as determined by the bioassay procedure, was used as antigen for the production of reagent antibodies. The protein was administered intradermally with Freunds complete adjuvant to rabbits. On days 21, 27, and 35, boosters were given. On day 37, the protein was given intravenously without any adjuvant. The whole schedule was monitored for antibody titre by an indirect ELISA which was previously standardized for optimal antigen coating concentration and working dilution of the goat antirabbit IgG-alkaline phosphatase conjugate.

Ouchterlony immunodiffusion test was performed to study antigenic relationships between different mosquito toxic strains of *B. sphaericus*, viz., 1593, B42,

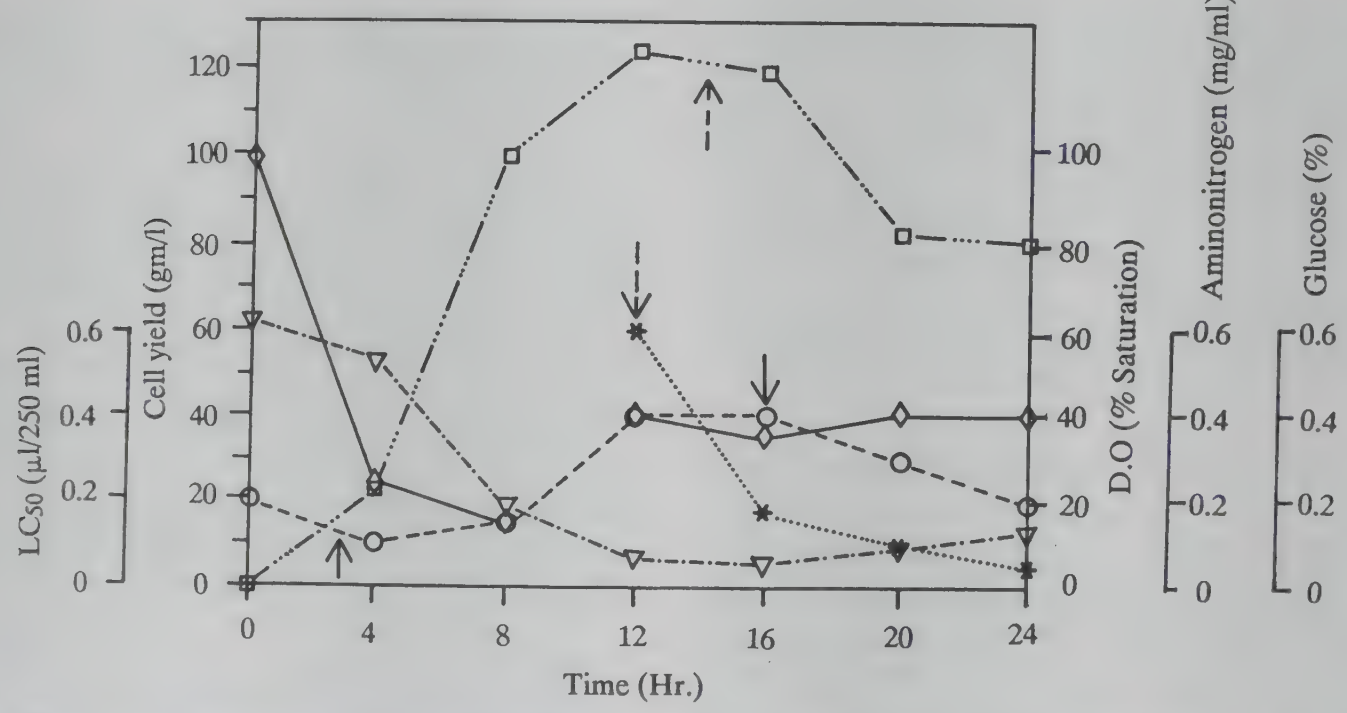


Fig. 4.2 The profile of cell yield (□), toxin synthesis (✱), glucose (○) and Aminonitrogen (▽)utilization and dissolved oxygen (◇) during the course of Fed-batch fermentation of *B.t* H.14.

↑ Glucose Feeding started ↓ Glucose Feeding stopped

↑ Beginning of sporulation ↓ Beginning of Toxin synthesis

2297 and a non-toxic strain (VCRC B 111) with the antiserum raised. When the antibody titres reached above 512×10^{-2}, the animals were bled and the serum separated. The serum was then dialysed, the IgGs were purified and the protein concentration was estimated. Protease activity, if any, in the purified immunoglobulin fraction was tested. Biotinylation of immunoglobulins was achieved using the Enzotin^{R} biotinylation kit which consisted of N-hydroxysuccinimidoester with a seven atom spacer. Standardization of the double antibody sandwich ELISA was done employing avidin- biotin system.

Two strains (1593 and B42) were grown in 2 l Erlenmeyer flasks containing NYSM medium with continuous shaking. Samples of culture were drawn at various time intervals and an aliquot was used to determine cell number. The toxin was extracted from the cells and the toxin quantity measured by ELISA as described above. Simultaneously, bioassays were carried out using lyophilized powder of whole cells. A simple correlation analysis was carried out to see the degree of relationship between toxin levels and Lc50 values for both the strains during various stages of growth.

The alkali solubilized fraction was separated on an anion ion-exchange column. Among the 5 fractions separated, only fraction number 2 showed larvicidal activity. SDS-PAGE analysis of this larvicidal fraction showed 3 bands corresponding to MW 43, 48, and 60 kDa.

Ouchterlony immunodiffusion showed that the antibodies reacted only with the alkali solubilized fractions of the *B. sphaericus* strains, 1593, B42 and 2297 as evidenced by precipitin lines and not with that of the non-toxic strain (B111). This suggests that though the strains are of different serotypes they share homology in the toxin (antigen) structure. This homology in toxin structure between strains of different serotypes can be taken to our advantage in that the ELISA can be used to measure toxin levels of toxic strains irrespective of their serotype.

The toxin levels and the LC 50 values during various stages of growth of the strains 1593 and B42 are given in Fig. 4.3. In the case of 1593, 43.0×10^{7} cells/ml were found to contain 4015 ng of toxin per ml in the 30th hr, whereas 8.5×10^{7} cells of B42 contained 4896 ng/ml. This shows that B42 synthesises more amount of toxin/cell.

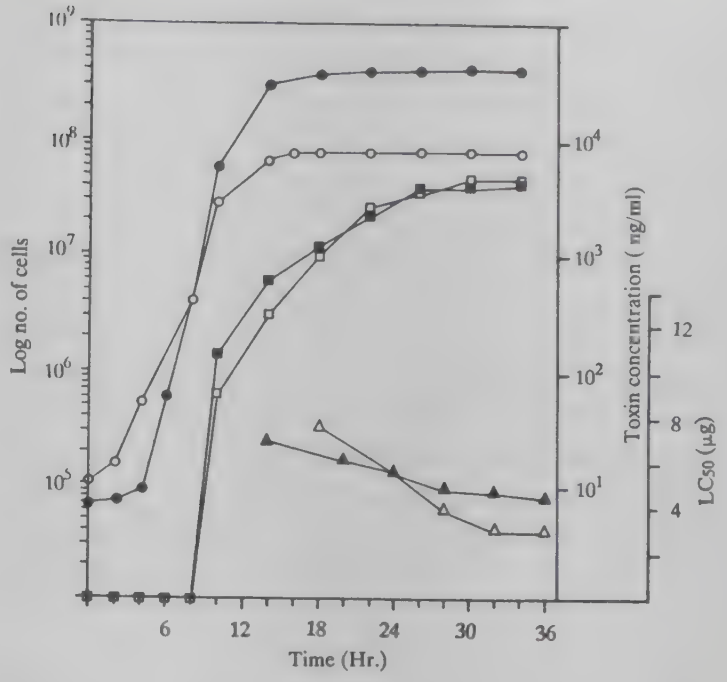


Fig. 4.3 Growth, toxin lvel and LC_{50} values of *B. sphaericus* strains, B42 and 1593.

The correlation observed between toxin levels as measured by ELISA and larvicidal activity as measured by bioassay was r = -.9898 and -.9887 for strains B42 and 1593, respectively. The correlation is highly significant (p = 0.05).

The ELISA developed using avidin-biotin amplification has a lowest detectable limit of 6.25 ng/ml. This is far superior to the ELISA using rabbit antitoxin IgG - alkaline phosphatase conjugate as the latter has a detectable limit of 40 ng/ml. Therefore the ELISA using avidin-biotin system has sufficient sensitivity to make it useful to monitor the toxin production during fermentation.

Safety evaluation of Bacillus sphaericus on mammals

B. sphaericus strain B42 was subjected to safety evaluation on mammals. Acute toxicity tests such as inhalation and dermal toxicity were carried out on rats and guinea pigs, respectively. These tests were done according to the principle of "maximum challange" as suggested by WHO with the dose of 2.8×10^{8} cells (10 mg lyophilised powder of biomass) per animal. It was observed that (in both inhalation and dermal toxicity studies) there was no mortality and all the animals were normal. Development of neither

erythema nor edema was observed in dermal toxicity. Food intake and weight gain by the treated animals were comparable to that of control animals. Gross examination of visceral organs did not reveal any abnormality.

Oesophageal procedure using rabbits was followed for mucous membrane irritancy test. The test material (0.5gm/animal) was fed to the animal through pharynx. Half the number of animals were sacrificed after 24 h and the rest after 48 h. The oesophagus and stomach were examined for signs of irritation such as erythema, edema, ulceration and mucous membrane sloughing as per Draize's scoring procedure. It was found that the animals were absolutely free from any sign of irritation.

Primary skin irritation test was performed on rabbits. Test material (500 mg per animal) was applied on intact and abraded sites. Then the skin sites were graded for primary skin irritation index as per Draize s scoring procedure, 24 and 72 h later. There was no dermal irritation and the primary irritation index value did not exceed 1^{+}.

Sub acute (90-days) oral toxicity was performed on rats. Rats were divided into four groups. Group I served as control and groups II, III and IV were administered orally 10, 20, and 40 mg, respectively of the test material, every day for 90 days. Animals were observed daily for development of symptoms for toxicity and mortality.

At the end of the treatment period the animals were sacrificed and the blood was collected directly from the jugular vein. Hematological (haemoglobin, red blood cell, white blood cell, platelet and reticulocyte counts) and biochemical (sodium, potassium, glucose, total protein, cholesterol, urea, creatinine, SGOT, SGPT and alkaline phosphatase) parameters were analyzed in blood and serum samples using standard procedures. Necropsy was conducted to find gross pathological changes in vital organs, visceral organs such as liver, kidney, heart, lungs, spleen, brain, adrenal and testes/ovaries. The organs were removed and their individual weight were recorded. Pieces of these organs were fixed in 10% buffered formaline for histopathological examination.

It was observed that there was no symptoms of poisoning and mortality in any of the groups of animals during the experimental period. The animals did not show any abnormality with respect to food intake, water consumption and weight gain. The absolute or relative organ weights of the animals did not reveal any abnormality. All haematological indices were within the normal range. The activities of the serum enzymes were normal and the results of the various bioconstituents in blood and serum were also within the limits of normal variation in all groups of treated animals indicating that there was no impairment of hepatic and renal functions. These findings suggests that *B. sphaericus* strain VCRC B42, when administered orally to rats for a period of three months up to 40mg (4 times maximum hazard level) per animal, did not have any adverse effect.

4.4. STUDIES ON *LAGENIDIUM* SP.

Identification of maintenance medium for new Lagenidium isolates

Four new isolates resembling *Lagenidium* sp. were obtained from soil samples and were designated as YC, THI, MK and BG. They were grown on 4 different media by seeding plates with zoospores. Samples were drawn daily and tested for zoospore production and larvicidal activity.

The results indicated that RGNSF medium favoured higher zoospore production as well as larvicidal activity much earlier in the *Lagenidium* isolate MK while soil extract medium did so in the case of THI and MK. Generally soil extract medium and RGNSF medium favoured higher zoospore production as well as enhanced larvicidal activity of all the *Lagenidium* isolates. Also these isolates retained the larvicidal activity at high levels for 9 days.

Comparative studies on the host range of new Lagenidium isolates

Studies were carried out on the host range of different *Lagenidium* isolates involving 7 mosquito species viz., *Cx. quinquefasciatus, Cx. sitiens, Cx. tritaeniorhynchus, An. subpictus, An. culicifacies, An. stephensi* and *Ae. aegypti*. The isolates were grown in PYGSF broth for 7 days at 100 rpm, on a rotary shaker. The biomass was harvested and 100 mg was bioassayed against different larval instars.

The results showed that in general, the new isolates of *Lagenidium* were highly active against the larval stages of different mosquitoes. Their activity

however was less against *Cx. quinquefasciatus*. Isolates YC, MK and BG exhibited decreased activity against late than early instar larvae of all the mosquitoes except *An. stephensi*. Against *An. stephensi* their activity increased with larval age. The activity of THI increased with the age of larvae of all the mosquitoes except *An. stephensi*, in which case it decreased.

Effect of vegetable oils on the growth and larvicidal activity of Lagenidium isolates

Vegetable oils such as sunflower oil, cotton seed oil and corn oil were explored as source of fatty acids for growing *Lagenidium* isolates. Various isolates were grown for 120 h in cotton seed broth supplemented with these oils, individually. Samples were drawn at 24 h intervals, biomass was separated, weighed and tested for zoospore production and larvicidal activity. It was observed that corn oil was better for the isolate YC, cotton seed oil for the isolate BG and sunflower oil and cotton seed oil for the isolates THI and MK.

Effect of lipids on the growth and larvicidal activity

Lipids are known to influence the growth, reproduction and infectivity of oomycetous fungi. Hence several fatty acids were investigated for their influence on the infectivity of the most active new *Lagenidium* isolate YC. It was grown in PYG broth supplemented with individual lipids for 96 h, along with appropriate controls. Samples were drawn at 24 h intervals and assessed for biomass production, zoospore production and larvicidal activity. The results indicated that cholesterol and triolein favour growth and zoosporogenesis leading to higher larvicidal activity.

Production of Lagenidium on lab and pilot scale

Attempts were made to produce the fungus (Isolate YC) on lab and pilot fermentors using RGNCSF medium. The fermentors (20 and 100 l capacity) were inoculated at 5% level and run with 1 l/min aeration and 110 rpm agitation at 30°C. The pH was set and constantly adjusted throughout the run at 6.5. Samples were drawn at 24 h intervals for 92 h and assessed for biomass yield, zoospore production and larvicidal activity. Three batches of the fungus were produced in each fermentor. The maximum biomass was produced after 72 h of growth in all cases. The biomass yield from different batches was in the range of 50-80 g/l, zoospore production 3-6 x 10^4/ml and larvicidal activity 30-40% (at 100 mg/250 ml).

Small scale field trial

A small scale field trial was carried out in pits containing seepage water located on the bank of a lake receiving sewage water. The pits had high level of organic pollutants and harboured *Cx. tritaeniorhynchus* and *An. subpictus* larvae. The fungus produced on RGNCF agar plates was applied to these pits as zoospores. Three applications were made at 2 months interval and immature density was monitored for 45 days before treatment and 260 days after the commencement of first treatment.

Before treatment the average density of different larval instars of culicines was 1.6-6.5 per 10 dips and that of pupae was 1.5 per 10 dips. On the day of treatment the density was 1, 4, 23, 17 and 3 of I,II,III, IV instars and pupae, respectively.The density of the immatures was reduced by 30-100% after 24 h of treatment and was maintained at the same level upto 63rd day post-treatment. The results indicate that the treatment of *Lagenidium* sp. exerted significant pressure on the culicine population in the pits containing seepage water. Similar trend was observed in the density of anophelines after the treatment of the fungus.

4.5. STUDIES ON THE MERMITHID NEMATODE *ROMANOMERMIS IYENGARI*

Effect of temperature on parasitic phase and postparasites

The effect of temperature on different developmental stages of the nematode was studied by incubating them at 20, 25, 30 and 35°C.

A smaller number of females with reduced length was produced at higher temperatures as compared to lower temperatures. This adverse effect considerably reduced their reproductive potential. It was observed that irrespective of incubation temperatures, majority of the male nematodes completed development and emerged first (during 0-12 h) from host larvae with higher parasite burden and there was a direct correlation between multiple parasitism and the number of larvae dying during this period. The females, especially those in hosts with single parasite, by and large required longer time to complete the development.

The period required for postparasitic juveniles to moult into adults was shorter at higher temperatures. It was 5 to 9 days at 35 °C and 7 to 14 days at 20°C. Likewise, at 35 °C the commencement of oviposition took 8 days and it lasted for 40 days. At 20 °C the commencement of oviposition took 19 days and oviposition lasted for 48 days. The mean number of eggs produced per female at 20 and 35° were significantly lower than that produced at 25 and 30°C.

Influence of soil moisture on the oviposition

Although most of the mermithids and their eggs cannot tolerate desiccation, recycling of *R. iyengari* in paddy fields has been reported. However, the mechanism of survival during dry season is not known. This was investigated by seeding postparasites of *R. iyengari* into 12 cm soil cores.

The data show that *R. iyengari* oviposited primarily in the upper 3 cm soil layer when the moisture level of the soil core was maintained at 15%. However, when it was allowed to dry, the nematode burrowed to the bottom of the soil core and oviposited. This study suggests, that *R. iyengari* withstands dry spells by burrowing deep into the soil where adequate level of moisture is present and at least a part of the eggs laid in the deeper soil strata may contribute for the recycling of the species when the habitats get flooded and mosquito breeding appears.

Influence of single and periodic flooding and harvest on productivity

The yield of eggs and preparasitic nematodes (ppn) and the infectivity of the ppn remained almost stable until the age of the culture reached 11-12 weeks and thereafter they declined very rapidly and ceased by 19th week. A linear decrease in infectivity of the ppn was noticed with increase in the number of flooding of cultures of different age groups. From the data obtained on different flooding schedules it can be concluded that the maximum yield of ppn can be obtained by flooding 5-7 week old cultures for 11 times at weekly intervals.

Susceptibility of different species of mosquitoes to infection and development

The susceptibility of different species and instars of mosquitoes to infection by the ppn of *R. iyengari* was studied by infecting laboratory reared larvae. All the 10 mosquito species tested were susceptible to *R. iyengari*, with certain degree of variations from species to species. Among culicines, *Cx. sitiens* was highly susceptible (susceptibility index : 1.1) at II instar followed by *Ae. aegypti, Ae. albopictus, Ar. subalbatus, Cx. tritaeniorhynchus* and *Ma. annulifera* and at IV instar, *Ar. subalbatus* was highly susceptible followed by *Ae. aegypti, Ae. albopictus, Cx. sitiens, Cx. tritaeniorhynchus* and *Ma. annulifera*. Among anophelines, *An. subpictus* was highly susceptible at II instar (susceptibility index : 0.7) followed by *An. stephensi* and *An. culicifacies* and at IV instar *An. stephensi* was highly susceptible (susceptibility index : 1.1) followed by *An. subpictus* and *An. culicifacies*.

The parasite burden was always higher in the larvae exposed at II instar than the ones exposed at IV instar except in the case of *Cx. tritaeniorhynchus* and *An. stephensi*. The highest parasite burden was observed in *Ae. aegypti* (average, 3.2), followed by *Ar. subalbatus* (average, 3.0), *An. culicifacies* (average, 2.5), *Ae. albopictus* (average, 2.2) and *Cx. quinquefasciatus* (average, 2.1). In the highest susceptible II instar larvae of *Cx. sitiens*, the parasite burden was only 1.3. Among IV instars, *Ar. subalbatus* had the highest parasite burden (average, 2.5), followed by *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* (average, 1.9) and *Ma. annulifera* (average, 1.1).

The duration of parasitic stage of *R. iyengari* in the host was 5-7 days, irrespective of the mosquito species and stage at which they were exposed to ppn. None of the imagoes from larvae exposed to ppn at II instar carried nematode parasites. Whereas, 1.0, 3.0, 5.0, 19.0 and 1.0% imagoes, respectively from *Cx. quinquefasciatus, Cx. sitiens, Ae. albopictus, Ae. aegypti* and *Ar. subalbatus* larvae exposed to ppn at IV instars carried the nematodes, none of the anopheline imagoes from larvae exposed to ppn infection carried nematodes.

4.6. STUDIES ON THE BY-PRODUCTS OF BIOPESTICIDES

Cyclosporine A

Immunosuppressive potential of Cyclosporine A

Pure Cyclosporine A from *Tolypocladium* sp. (by a process developed at VCRC) was evaluated for immunosuppressive potential by skin grafting in rats along with the commercially available Sandimmune

(M/s. Sandoz Ltd.). The drug was tested at 15 and 30 mg/kg/day for a period of 14 days. The animals were monitored for graft acceptance and alterations in biochemical parameters such as serum glutamate oxaloacetate transferase (SGOT), glutamate pyruate transferase (SGPT), creatinine and blood urea. Circulating Cyclosporine A levels were monitored by HPLC using a C18 column.

The Sandimmune treated animal group showed 83% acceptance of the skin grafts while the test preparation treated group showed 67-83% acceptance. The degree of nephro- and hepatotoxoicity was comparable for both the preparations. The results of this study show that the process employed in the purification of Cyclosporine A does not affect the biological activity of the compound.

Bioreactor system for the production of Cyclosporine A

Immobilisation of *Tolypocladium* sp. in a 2% biopolymer was reported in 1989 annual report. Studies were carried out to increase the yield of Cyclosporine A by recycling the substrate and also by studying the influence of precursor amino acids on the yield.

It was found that recycling of the substrate for three cycles increased the yield of Cyclosporine A from 320 mg/ml to 600 mg/ml. The recycling mode promotes the better utilisation of the substrates and increases the yield.

Immobilisation of the spores was also done and the mycelium allowed to grow inside the biopolymer beads and used for the biosynthesis of Cyclosporine A in a fluidised bed reactor in recycle mode. The yield of Cyclosporine A was comparable to that obtained by immobilizing the vegetative mycelium as such.

The half-life of the immobilised system was estimated to be 5-7 months. As an immobilised system can be used for 3-4 half-life periods there is every possibility of using this system for 1.5-2 years.

The effect of leucine and valine individually and in combination, on the synthesis of Cyclosporine A under immobilised condition was studied . It was found that by increasing the concentration of either leucine or valine from 750 mg/l to 2g/l the yield of Cyclosporine A increased nearly 15 times. However the addition of both leucine and valine together did not affect the yield when compared to the yield obtained by the addition of either of the amino acids. Though the entire amount of amino acids added in the medium is not utilised for Cyclosporine A synthesis, their addition enhances the synthesis by acting as a promotor.

Enzyme systems involved in synthesis of Cyclosporines

Studies on the enzyme systems involved in Cyclosporine synthesis were initiated in order to get better insight on the mechanism of synthesis. Two enzymes, Methionine Activating Enzyme (MAE)which synthesises S-Adenosyl Methionine (SAM), the methyl donor for the amino acids, required for Cyclosporine synthesis and Cyclosporine synthesising multienzyme complex were studied in detail and characterised. It was found that the specific activity of MAE increases during the growth of the mycelium with corresponding increase in the total Cyclosporine levels indicating a positive role for MAE in the synthesis. MAE is involved in the synthesis of SAM which acts as a methyl donor for the methylation of aminoacids in the Cyclosporine molecule. This enzyme was purified using standard chromatographic procedures and characterised. The MW of this enzyme was 18kDa and required the presence of Mg^{++} for its activity. Mn^{++} restored the activity partially in the absence of Mg^{++}. The enzyme had a pH optimum of 8.0 and a temperature optimum of $37^{0}C$. Sodium fluoride and EDTA inhibited the enzyme activity completely indicating the metal ion requirement of the enzyme. Iodoacetic acid inhibited the activity by 33% indicating the -SH requirement of the enzyme.

The purified Cyclosporine synthesising enzyme complex had a MW of 720 kDa. The enzyme activity was determined by estimating the thiol binding of ^{14}C-leucine to the enzyme complex. Iodoacetic acid inhibited the enzyme activity and methylleucine did not compete with leucine for the binding sites on the enzyme complex.

Methyltransferase activity in the complex was estimated in the complex by estimating the conversion of the aminoacids to their corresponding methyl amino acids. It was found that the methyl aminoacids were bound to the complex and not detected in the supernatant. Amino acids and methyl amino acids were measured using a HPLC after derivatisation with phenylisothiocyanate.

At no stage in the growth of the mycelium, methyl amino acids were detected. This along with the studies on enzyme complex suggest that the amino acids of Cyclosporine bind to the enzyme complex through thiol groups, where they are methylated followed by peptide growth and cyclisation to synthesise the complete Cyclosporine molecule.

L-Dihydroxy phenyl alanine (L-dopa)

In order to understand the basic parameters governing the production of L-dopa by *B. thuringiensis* MB24 attempts were made to purify the enzyme Tyrosine hydroxylase which catalyses the conversion of L-tyrosine into L-dopa and characterize its activity.

The bacterium was grown in NYSM medium containing L-tyrosine in a 100 l fermentor. The biomass was removed and the filtrate was passed through ultra-filters of different molecular weight cut-off. The ultrafiltration retentate of each step was tested for tyrosine hydroxylase activity. Retentates of more than 1,00,000 mw and more than 1,000 mw but less than 10,000 mw cut-off filtration showed high activity.

The activity of crude enzyme was characterized in relation to different parameters such as pH, temperature, metal ions and inhibitors. The optimum temperature and pH level were 30°C and 7.5, respectively. Among different ions tested Zn^{++} resulted in enhanced activity. EDTA, sodium fluoride and iodoacetic acid inhibited the activity.

5. INSECTICIDES

5.1. DEVELOPMENT (SYNTHESIS & FORMULATIONS)

5.1.1. **Pyrethroid esters:**

A wide range of pyrethroid insecticides can be synthesised by reacting suitably substituted acids as well as the alcohols for enhancing biological activity. In the present study, different substituted 2-phenoxy-3-methyl butanoic acids were prepared and reacted with three alcohols, viz., 3-phenoxy benzyl alcohol, α-cyano-3-phenoxy benzyl alcohol and 3,4-methylenedioxy benzyl alcohol (piperonyl alcohol) to study the structure-activity relationship in the resulting esters.

The 2-phenoxy-3-methyl butanoic acids were prepared by refluxing the different phenols with 2-bromo-3-methyl butanoic acid (α-bromo isovaleric acid) in 60 - 80% yield. The reaction of the respective acid chloride with the benzyl alcohol was carried out in benzene using pyridine base to produce the corresponding 2-phenoxy-3-methyl butanoates in 40 - 95 % yield. The crude esters were purified to give the racemic esters as colourless to pale yellow viscous liquids by column chromatography using 5% ethyl acetate - petroleum ether as eluent. Altogether thirty-nine esters were synthesised. The IR, ^{1}H-NMR spectral data coupled with the mass spectral data were in full agreement with the structures of the esters.

The different 2-phenoxy-3-methyl butanoates synthesised as racemic esters were evaluated for both larvicidal and adulticidal activities against *Cx. quinquefasciatus*, the filariasis vector. The structure-activity relationship deals with the effect of (i) different substituents in the acid moiety; (ii) their relative positions and (iii) three different alcohols used in the esterification in influencing the biological activity of the resulting 2-phenoxy-3-methyl butanoates.

The results of the larvicidal and adulticidal activities of the effective esters against the respective fourth-instar larvae and unfed females of *Cx. quinquefasciatus* are presented in Table 5.1.

Considering the biological activity of 3-phenoxybenzyl esters with dif-ferent substituents in the 4-position of the phenyl ring in the acid moiety, the larvicidal activity was found to increase in the following order: isopropyl < tert. butyl < bromo < chloro < methoxy < fluoro whereas the adulticidal activity was found to increase in the order: isopropyl < tert.

Table 5.1.

Larvicidal and adulticidal activities of effective 2-phenoxy-3-methyl butanoates.

Code No.	R^1	R^2	R^3	LC_{50} (mg/l)	LD_{50} (μg/insect)
P6	4-t.Bu	CN	3-OPh	0.2326	0.0091
P17	4-OMe	H	3-OPh	0.0146	0.0034
P22	4-Br	H	3-OPh	0.0515	0.0014
P24	2-Cl	H	3-OPh	16.2502	0.0094
P25	2-Cl	CN	3-OPh	0.8709	0.005
P27	3-Cl	H	3-OPh	5.1075	0.0023
P28	4-Cl	H	3-OPh	0.0208	0.0034
P29	4-Cl	CN	3-OPh	0.0114	0.0003
P35	3-F	H	3-OPh	0.2942	0.0013
P37	4-F	H	3-OPh	0.0139	0.0083
P38	4-F	CN	3-OPh	0.0025	0.0049
P39	4-F	H	3,4-OCH_2O	0.0159	0.0124
Fenvalerate (Standard)				0.0028	0.0001

butyl < fluoro < methoxy < chloro < bromo.

The position of the substituent was also found to influence the biological activity. Among the different 2-, 3- and 4- substituted phenoxybenzyl esters, larvicidal activity was found to increase in the order 2- < 3- < 4- (Fig 5.1). But there was no uniform relationship with respect to position of the substituent in the case of adulticidal activity. The 3-substituted esters were more effective in the case of fluoro and chloro esters followed by substitutions in the 4- and 2- positions. The 4- substituted esters were effective in the case of bromo and methoxy esters (Fig 5.2).

The effect of disubstitution was found to reduce both

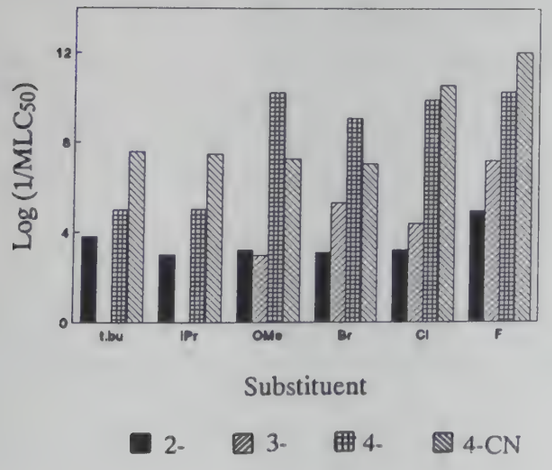


Fig. 5.1 Effect of substituent position on the larvicidal activity.

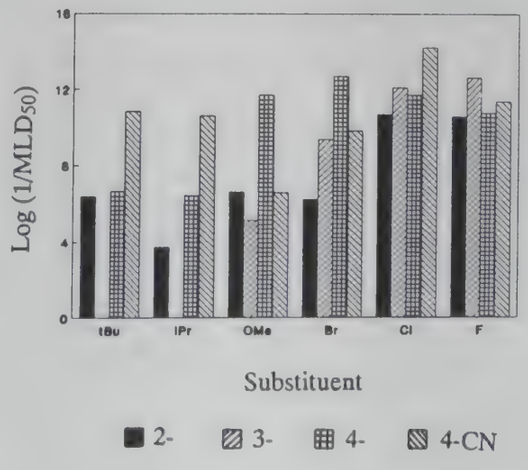


Fig. 5.2 Effect of the substituent position on the adulticidal activity.

larvicidal and adulticidal activities of both 2,6-dis ubstituted tert. butyl ester and 2,4-disubstituted chloro ester compared to the respective 2- and 4- substituted esters.

Among the three types of benzyl esters, 3-phenoxy benzyl esters were found to be more effective than 3,4-methylenedioxy benzyl esters for both larvicidal and adulticidal activities. The introduction of α-cyano group in the 3-phenoxybenzyl esters with substituent in the 4-position was found to increase both larvicidal and adulticidal activities in the case of tert.butyl, isopropyl, chloro and fluoro esters and reduce the activity in the case of methoxy, and bromo esters. (Figs. 5.1 & 5.2)

Out of thirty-nine 2-phenoxy-3-methyl butanoates synthesized and tested for insecticidal activity against *Cx. quinquefasciatus*, six compounds (P17, P22, P28, P29, P37 & P39) were found to be effective with LC_{50} values ranging between 1.14 and 5.15×10^{-2} mg / l and ten compounds (P6, P17, P22, P24, P25, P27, P28, P35, P37 & P38) were found to be effective for adulticidal activity against *Cx. quinquefasciatus* with LD_{50} values ranging between 1.3×10^{-3} and 9.4×10^{-3} ug / insect. It was observed that P38, a-cyano-3-phenoxybenzyl- 2-(4-fluorophenoxy)-3-methyl butanoate with an LC_{50} value of 2.5×10^{-3} mg/l and P29,α-cyano-3-phenoxybenzyl-2-(4-chlorophenoxy)-3-methylbutanoate with a LD_{50} value of 3.0×10^{-4} ug / insect were comparable in their activities to that of fenvalerate, a well known non-cyclopropane pyrethroid ester (LC_{50} = 2.82×10^{-3} mg / l; LD_{50} = 1.37×10^{-4} ug/insect).

Quantitative structure activity relationships (QSAR):

Biological activity has been known to be influenced by one or more physicochemical substituent parameters like lipophilicity, electronic and steric factors. To understand the structural factors determining the variations in the biological activity of a set of 3-phenoxybenzyl, a-cyano-3-phenoxybenzyl and 3,4-methylenedioxy benzyl-2-phenoxy 3-methyl butanoates, a quantitative analysis of the larvicidal and adulticidal activities against *Cx. quinquefasciatus*, the filariasis vector in terms of the substituent effects using physicochemical substituent parameters was carried out. A multiple regression analysis was undertaken to gain insight into the gross properties affecting insecticidal activity and to derive structure activity relationships which may be of assistance in designing more potent analogues.

The QSAR studies showed that the biological activity was influenced by a combination of two or all the three physicochemical substituent parameters. In the case of 3-phenoxybenzyl esters, lipophilicity and electronic factors were found to play a role whereas for α-cyano-3-phenoxybenzyl esters, lipophilicity, electronic and steric factors were found to influence the biological activity. The biological activity of 3,4-methylenedioxybenzyl esters was influenced by lipophilicity and steric factors. All the relationships stated above were significant at the 95% level.

The octanol/water partition coefficient (log P) values and the insecticidal activity expressed as log (1/MLC50) or log (1/MLD50) of the three types of esters were analysed for the influence of log P on the biological activity. In both cases, there was a parabolic relationship existing between the log P and the larvicidal and adulticidal activities. This clearly shows that in this series of compounds, an optimum value for log P ranging between 5.4 and 6.2 is important for realizing the maximum biological activity.

5.1.2. Controlled release formulations of a repellent:

Three controlled release formulations of the insect repellent, DEPA (N,N-Diethyl phenyl acetamide) - Depa-A, Depa-B and Depa-C have been developed and tested on human subjects against *Aedes aegypti* adult mosquitoes for repellency. These formulations were made with a pharmacologically safe and hydrophilic polymer as additive and ethanol as the vehicle. The concentration of the repellent was made as 20% in all the formulations and the level of polymer concentration was 0, 5, 10 and 20% in formulations Depa-0, Depa-A, Depa-B and Depa-C respectively in ethanol.

The formulations were tested for repellency in a colony cage (55 x 55 x 55 cm) against three to four day old unfed *Aedes aegypti* adult mosquitoes after treating the fore arms with the repellent formulations. Percentage increase in protection time of the polymer formulations with respect to Depa-0 was calculated as,

$$\text{Increase in P.T.}(\%) = \frac{(T-C) \times 100}{C}$$

Where T is the protection time of the formulation with the polymer additive and C is the protection time of the formulation Depa-0, without the polymer additive. The testing was carried out at 27 $\pm$ 2°C with the relative humidity ranging from 75 to 90%.

The protection times at the two application rates of 0.5 mg/cm^2 and 0.25 mg/cm^2 with Depa-A were found to be 6.63 and 5.75 hr with the respective percentage increase of 34.2 and 29.2 over Depa-0. Whereas there was a considerable increase in the protection time with Depa-B with the respective values of 7.13 and 6.15 hr with the percentage increase of 44.3 and 38.2 compared to Depa-0. The treatment with formulation Depa-C was found to give white deposit on the treated skin at the application rate of 0.5 mg/cm^2 which might not be acceptable to any user. Whereas in the treatment with Depa-A and Depa-B, there has been no white deposit on the treated area. Moreover, Depa-B application does not show any oily appearance on the treated skin as partial oily appearance has been observed with the treatment in the case of Depa-A.

Standardization of a method for determining repellent concentration using IR spectrophotometer:

The intensity of absorption of carbonyl function at 1630 cm^{-1} in an Infra-red spectrophotometer has been found to be proportional to the concentration of the repellent. IR spectra were run for known concentrations of the repellent in chloroform. A linear relationship obtained by plotting the inverse of (100-% Absorption) against the concentration was used for the quantitative analysis of unknown concentration of the repellent.

Evaporation studies of the formulations Depa-0, Depa-A and Depa-B were carried out from two different surfaces, one adsorbing (Whatman No.1 filter paper) and the other non-adsorbing, (a tracing paper) under accelerated conditions of higher temperature. The formulations were applied uniformly through out the area of the paper strips of size 1 X 5 cm at the application rate of 500 µg/cm^2 of the repellent. The treated paper strips were kept in an air oven at 60°C and strips were withdrawn at different intervals and the concentration of the repellent on each type of paper surface at different intervals was obtained from the standard graph.

Comparison of protection time of different formulations was carried out by one way analysis of variance (ANOVA) at level of significance 0.05. A linear regression of quantity of repellent to time was followed to find out the slope of the evaporation curve for different formulations. The magnitude of the X-coefficient of the regression equation was taken as the slope.

The evaporation studies at accelerated conditions from the two types of surfaces showed two different patterns (Figures 5.3a & 5.3b; 5.4a & 5.4b). The results of evaporation from the non-adsorbing surface showed that even among the polymeric formulations, repellent evaporation from Depa-B was less compared to that of Depa-A (Fig 5.3a), which again justifies the higher protection time observed with the

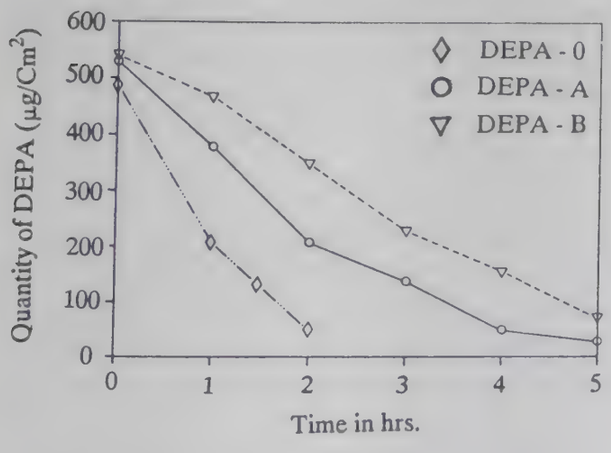


Fig. 5.3a **Evaporation of the repellent from non-adsorbing surface model.**

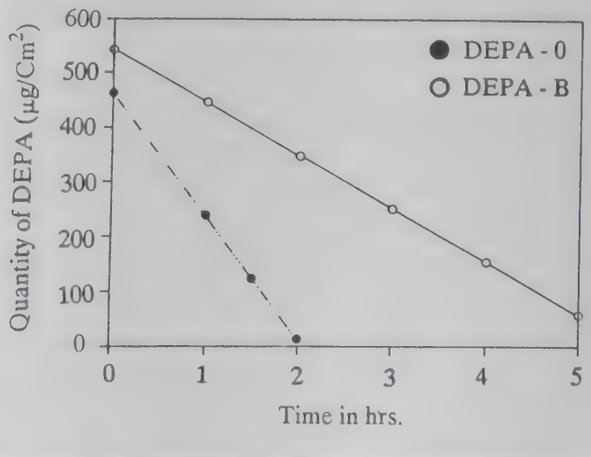


Fig. 5.3b **Regression of evaporation of repellent on time from the non-adsorbing surface model.**

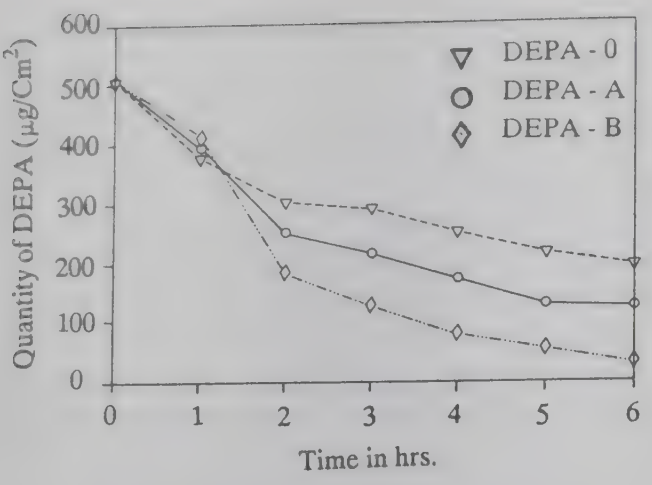


Fig. 5.4a Evaporation of the repellent from the adsorbing surface model.

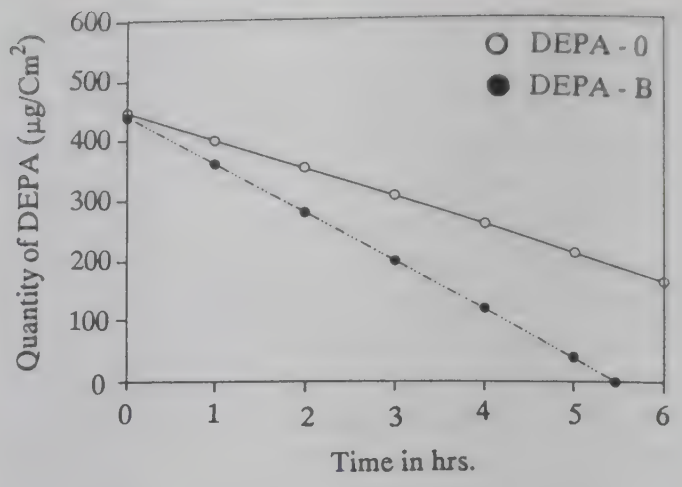


Fig. 5.4b Regression of evaporation of repellent on time from the adsorbing surface model.

testing of the formulations on the human skin. The results of the evaporation of the repellent from the adsorbing surface showed that the evaporation of the repellent from Depa-A and Depa-B formulation was higher compared to the Depa-0 formulation. The possible explanation for these observations is that the polymer physically binds with the repellent molecule and prevents normal evaporation and adsorption.

The concentration of the polymer was found to influence the protection time as well as the cosmetic acceptability and an optimum level of the polymer additive was found to be appropriate, beyond which the addition of polymer may reduce the protection time as well as the cosmetic acceptability. Based on these observations Depa-B was found to be promising with improved protection time and cosmetic acceptability.

5.1.3. Controlled release formulation of a mosquito larvicide:

Conventional application of larvicides to control the breeding of mosquito vectors in different habitats involves periodic treatment which results in high and possibly toxic concentrations in the aquatic environment which subsequently diminishes below the minimum effective level in time. A slow release formulation if effective would be ideal in such situations for increasing the active life of the larvicides which would bring down the cost of application by reducing the frequency of application as well as minimize the pollution to the environment.

Sodium carboxymethylcellulose is a water soluble polysaccharide derivative with excellent gel-forming, thickening and suspending properties making the solution in water ideal for many industrial applications. The formulations were prepared in a neutral aqueous solution of the polymer with two different concentrations of fenthion with respect to dry weight of the polymer in the final formulations. Low density materials were incorporated to make the formulations suspend in water. Dried slabs were made after incorporation of the active ingredient (ai) into 20% aqueous solution of sodium carboxymethylcellulose followed by subsequent drying under shade for 4 - 5 days. The incorporation of the ai was made to give 20% and 30% with respect to the dry weight of the slab. The crosslinking of the dried slabs was carried out with 1.0 M solution of Copper sulphate. Formulations with a cross-linking extent of 24 and 48 hours were developed. Thus formulations F_1 and F_2 containing about 2 g of ai (20% with respect to dried formulated material) and F_3 and F_4 with 3 g of ai (30% with respect to dried formulated material) were developed at two crosslinking periods of 24 and 48 hr respectively.

Release kinetics:

The formulations were placed in a glass trough containing 5 liters of water. The release profile was monitored at the static conditions of water at room temperature ($29 \pm 3^{o}C$) by withdrawing a measured volume of water and a standard colorimetric procedure was followed to estimate the concentration of the insecticide. The water in the troughs was changed at 24 hr intervals and before changing the water, the slabs were taken out with the help of a strainer and the water samples were collected for analysis of fenthion concentration. The evaluation of the formula-

tions was continued till the formulations started eroding.

Cross-linking for a period below 24 hours was found inadequate to meet the stability requirements. Formulations with a crosslinking extent of 24 and 48 hours were found to give appreciable stability in water. The release profile of the formulations F_1 and F_2 are given in figures 5.5a and 5.5b which show the weekly release as well as the cumulative concentration of the fenthion release at weekly intervals of time. These formulations were found to be intact for a period of 25 weeks after which the formulations started eroding. The release profile of the active ingredient from the formulations F_3 and F_4 are given in figures 5.6a and 5.6b. The analysis of these formulations was discontinued after 14 weeks as they started eroding.

Formulation F_1 has been found to release the ai at an average rate of 6.87 mg/week. A similar trend was observed with the formulation F_2 with the average release rate of 6.38 mg/week. The average release rates were found to be 17.66 and 15.98 mg/week respectively for formulations F_3 and F_4. The formulation F_2 was found to be quite stable and the release rate was maintained very well compared to F_1. The concentration of fenthion ranged between 0.07 and 0.21 mg/l at the application rate of one slab per five litres of water. Therefore the formulation with a 20% larvicide concentration and cross-linking period of 48 hours was found to be appropriate. The higher extent of cross-linking gave sufficient stability and uniform release of active ingredient.

The comparison of the release profile of the four formulations showed that formulations with cross-linking period of 48 hours were found to give an appreciable stability as well as a steady release rate. The F_2 formulation after the initial period of 3 weeks, was found to release the ai at a sustained rate of 3 - 4 mg/week from 4th to 22nd week showing that the

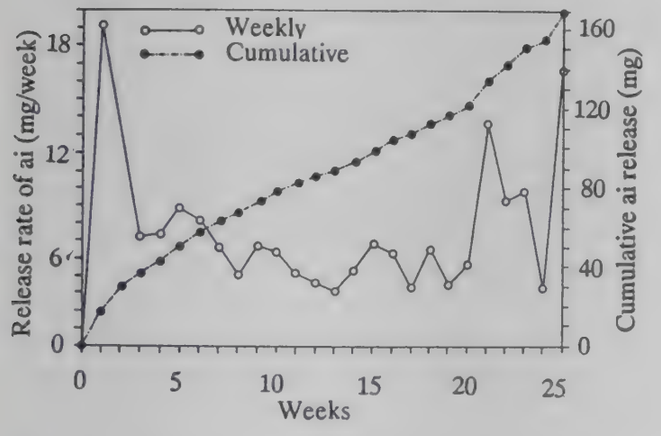


Fig. 5.5a Release profile of formulation F_1

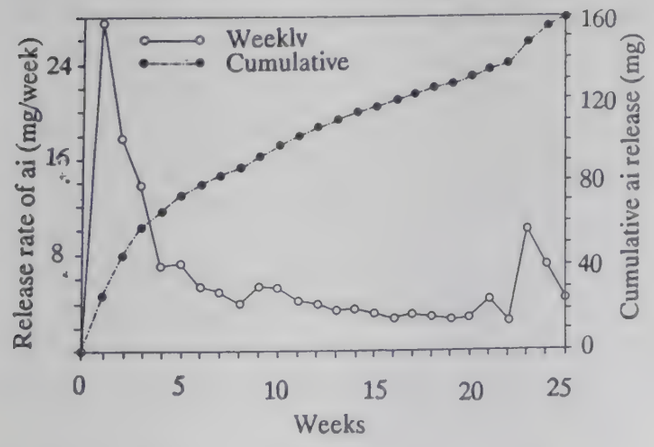


Fig. 5.5b Release profile of formulation F_2

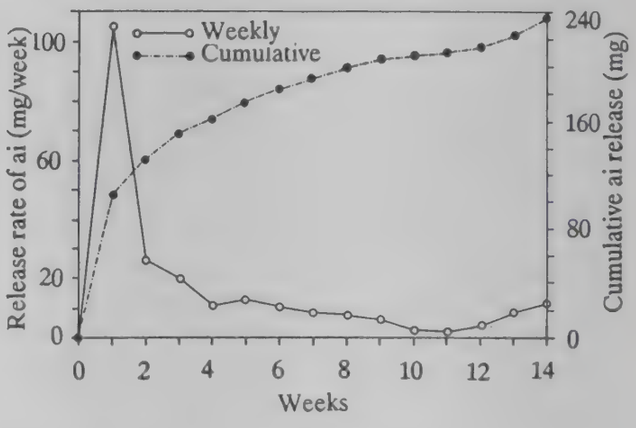


Fig.5.6a Release profile of formulation F_3.

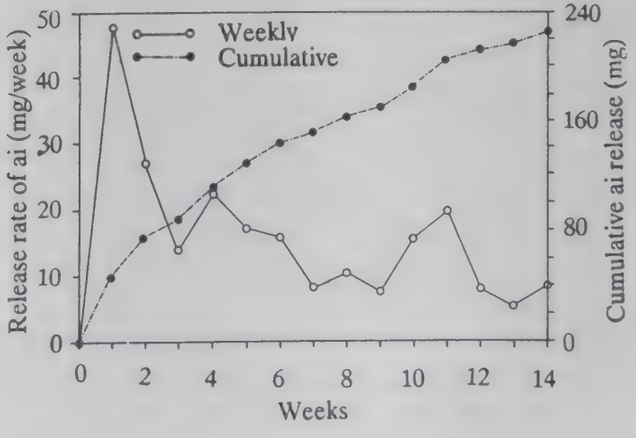


Fig. 5.6b Release profile of formulation F_4

release profile is independent of the ai load in the formulation, thus a zero order release kinetics. This is an ideal system for the application as mosquito larvicide. In the field situations, the initial higher dose may bring down the population to an appreciable level and the insecticide loss through the environmental degradation will be compensated by the ai release during the subsequent period.

The gelled carboxymethylcellulose with copper salt can be utilized as a controlled release monolithic device for increasing the active life of a mosquito larvicide. This system also satisfies the conditions of the recent requirements of a controlled delivery system as it is biodegradable, economical, easy to process at ambient conditions.

5.2. WORLD HEALTH ORGANIZATION PESTICIDE EVALUATION SCHEME (WHOPES):

5.2.1. Determination of adult and larval susceptibility of laboratory colony mosquito species to insecticides:

Discriminating dosages of the following insecticides were determined in order to study the susceptibility status of laboratory reared mosquito species by using standard WHO procedures (WHO, 1981, 1982).

Adulticides

1. Malathion	...	5.0 %
2. Deltamethrin	...	0.025 %
3. Permethrin	...	0.25 %
4. Propoxur	...	0.1 %
5. Fenitrothion	...	1.0 %

MILarvicides

1. Fenthion	...	82.5%
2. Malathion	...	50.0%
3. Temephos	...	50.0%
4. Deltamethrin	...	0.02%
5. Alphamethrin	...	10.0%

The species tested were *Culex quinquefasciatus, Cx.sitiens, Cx.tritaeniorhynchus, Aedes aegypti, Ae.albopictus, Anopheles stephensi, Armigeres subalbatus* and *Toxorhynchites splendens*. The insecticide impregnated papers were obtained from WHO for determining the critical exposure period to adults. Critical doses for the larvicides were also determined against the above mentioned mosquito species. From the percentage mortalities obtained at different concentrations in larvae and those obtained at different exposure periods in adults, by using probit analysis, critical dose and critical exposure time were calculated and expressed as $LC_{99.9}$ (mg/l) and $LT_{99.9}$ (min) respectively.

The susceptibility status of fourth-instar larvae and fully fed adults of various mosquito species of colony population is presented in Table 5.2. The adults of *An. stephensi* and *An. culicifacies* were more susceptible to propoxur and deltamethrin respectively whereas those of *Cx. sitiens* were more susceptible to malathion, fenitrothion and permethrin than other species when $LT_{99.9}$ of various mosquito species were compared.

On screening $LC_{99.9}$ of various mosquito species, it was evident that the larvae of *Cx. quinquefasciatus, Ar. subalbatus* and *Cx. tritaeniorhynchus* were comparatively more susceptible to fenthion and alphamethrin, whereas larvae of *Cx. sitiens, Cx. tritaeniorhynchus* and *Ar. subalbatus* showed higher susceptibility to temephos, malathion and deltamethrin respectively. From the analysis of the data it was observed that the larval and adult populations of many mosquito species were heterogeneous with high chi square value.

5.2.2. Effect of a new synthetic pyrethroid, β-Cyfluthrin, against vector mosquitoes:

β-Cyfluthrin, a new synthetic pyrethroid has been evaluated for its larvicidal and adulticidal efficacy against various mosquito species (Table 5.3). To this synthetic pyrethroid at 2 ug/cm^2, the adults of *An. culicifacies* were the most susceptible (LT_{50} = 27.76 min) whereas those of *Ae. aegypti* were the least susceptible (LT_{50} = 39.92 min). The larvae of *Ar. subalbatus* were highly susceptible, with LC_{50} value of 7.76×10^{-7} mg/l which was 1,37,500 times lesser than that of the least susceptible *An. stephensi* (LC_{50} = 0.116 mg/l). Malathion resistant strain of *Cx. quinquefasciatus* was about four times more tolerant, whereas the strain resistant to both malathion and fenthion was equally susceptible as the normal laboratory reared strain.

The residual efficacy of this pyrethroid was determined on different surfaces viz. cement, asbestos,

Table 5.2

Adult and larval susceptibility status of mosquitoes of colony population to selected insecticides.

Test species	Malathion	Fenitro thion	Propoxur	Permeth-rin	Deltameth-rin
			Adults : $LT_{99.9}$ (min)		
Cx. quinquefasciatus	275.59	1806.37	7926.37	999.99	216.55
Cx. tritaeniorhynchus	150.71	1623.57	254.12	347.62	206.16
Cx. sitiens	106.79	257.31	1345.92	77.05	129.73
Ae. aegypti	281.03	380.69	1952.18	108.94	90.40
Ae. albopictus	217.60	409.40	644.33	1419.81	92.59
An. stephensi	168.01	367.69	139.57	144.32	98.56
An. culicifacies	429.51	1123.49	464.52	296.18	54.90
Tx. splendens	172.24	395.63	427.45	231.42	813.70
	Malathion	Fenthion	Temephos	Alpha-methrin	Delta-methrin
			Larvae : $LC_{99.9}$ (mg/l)		
Cx. quinquefasciatus	0.1783	0.0132	0.00082	0.00018	0.00034
Cx. tritaeniorhynchus	0.1095	0.0138	0.00034	0.00017	0.00056
Cx. sitiens	0.1705	0.0142	0.00021	0.00205	0.00153
Ae. aegypti	1.0718	0.1017	0.00198	0.00203	0.00219
An. stephensi	1.3353	0.1410	0.01143	0.00826	0.03672
An. culicifacies	0.9185	0.0259	0.00121	0.00140	0.02290
Ar. subalbatus	0.3306	0.0134	0.00059	0.00017	0.00017
Tx. splendens	1.5161	0.0578	0.00091	0.01620	0.01163

thatch and mud at the application rate of 50 mg(ai)/m^2 against different mosquito species. It was found that the efficacy persisted in all the treated surfaces except mud against *Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. sitiens, Ae. aegypti, Ar. subalbatus* and *An. culicifacies*, resulting in more than 85% mortality. About 70-95% mortality was observed in *An. stephensi* only for 2 weeks. However, this compound did not show any residual effect on any of the treated surfaces against *Tx. splendens*. Further evaluation is in progress.

5.2.3. **Evaluation of bioallethrin mats using different heating devices against mosquito vectors:**

Bioefficacy:

The bioefficacy of the bioallethrin mats was assessed in a Peet-Grady chamber ($8m^3$). The tests were conducted against laboratory reared *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti*. The mat was heated throughout the test period of 10 hr. Batches of 100 unfed female mosquitoes were released at hourly intervals and after 1 hr exposure, the number of mosquitoes knocked down, alive and dead were collected and kept in a holding cage with glucose pad for observing the mortality after 24 hr. The experiments were conducted from 7.00 p.m. to 5 a.m. for *An. stephensi* and *Cx. quinquefasciatus* and from 7 a.m. to 5 p.m. against *Ae. aegypti*. The tests were also

Table 5.3.

Larvicidal and adulticidal efficacy of β-cyfluthrin against mosquito species.

Species	LC_{50} (mg/l)	LC_{90} (mg\l)	Regression	χ^2
		Larvae		
Cx.quinquefasciatus	$5.62X10^{-5}$	$9.19X10^{-5}$	30.46 + 2.60 lnX	2.554
Cx.quinquefasciatus (M. Res.)	$2.04X10^{-4}$	$7.07X10^{-4}$	13.76 + 1.03 lnX	5.909
Cx.quinquefasciatus (M. & F.Res.)	$8.13X10^{-5}$	$4.48X10^{-4}$	12.06 + 0.75 lnX	1.547
Cx.tritaeniorhynchus	$1.93X10^{-4}$	$3.63X10^{-4}$	22.27 + 1.93 lnX	2.376
Cx.sitiens	$1.49X10^{-4}$	$1.68X10^{-3}$	9.66 + 0.53 lnX	2.786
Ae.aegypti	$1.19X10^{-4}$	$2.12X10^{-4}$	25.16 + 2.23 lnX	16.578
An.stephensi	0.11603	0.31596	7.63 + 1.17 lnX	6.952
An.culicifacies	$5.51X10^{-3}$	0.47491	6.49 + 0.29 lnX	3.378
Ar.subalbatus	$7.76X10^{-7}$	$1.66X10^{-7}$	6.49 + 0.29 lnX	8.606
Tx.splendens	$1.98X10^{-4}$	$5.21X10^{-4}$	16.78 + 1.32 lnX	18.223
		Adults		
	LT_{50}(min)	LT_{90}(min)	Regression	χ^2
Cx.quinquefasciatus	33.05	50.93	-5.35 + 2.95 lnX	5.286
Cx.tritaeniorhynchus	31.29	53.40	-3.24 + 2.39 lnX	21.021
Ae.aegypti	39.92	60.27	6.46 + 3.11 lnX	0.567
An.culicifacies	27.76	45.72	-3.52 + 2.56 lnX	13.989
An.stephensi	34.51	78.90	-0.48 + 1.55 lnX	16.179
Ar.subalbatus	34.52	49.94	-7.27 + 3.47 lnX	6.611
Cx.sitiens	37.48	55.40	-6.87 + 3.28 lnX	12.791

carried out on different days with different heating devices. The evaluation was carried out with unfed mosquitoes and with a host (chicken/rabbit) inside the chamber to find out the protection offered due to heating of mats. There was an exit trap connected to the chamber to find out any excito-repellency due to the released bioallethrin from mats.

Analysis of bioallethrin in mats at different periods of heating with different heating devices:

Individual mats were used in different heating devices and after known periods of heating, i.e., 1, 2, 4, 12 and 24 hr, each mat was extracted with acetonitrile and the extract was analysed for the concentration of bioallethrin using high performance liquid chromatography (HPLC) and a UV detector at 230 nm with a 0.01 AUFS sensitivity. Acetonitrile: Water (with 0.1% phosphoric acid) in the ratio of 70 : 30 was used as a mobile phase at the flow rate of 2 ml/min and a C-8 RP column was used for the analysis.

The results showing the percentage knockdown and mortality against the three vector mosquito species, *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* with different heating devices during the heating period of 10 hr are presented in Table 5.4. The results showing the amount available and release rate of bioallethrin in mats at different periods of heating with different heating devices are shown in Figures 5.7 and 5.8.

The results of the bioassay tests with bioallethrin mats in the Peet-Grady Chamber using different heating devices indicate that there was appreciable knockdown of all the species tested throughout the test period of 10 hr. However, the percentage mortalities observed were not appreciable in all cases.

The testing of the devices against *Cx. quinquefasciatus* showed that devices B and C were found to produce significant mortality whereas the devices A and D could show only percentage mortalities less than 35 %. Against *An. stephensi*, except the device A, all other devices were found to be effective. All the four devices were found to be effective against *Ae. aegypti*.

Another study to find the availability of bioallethrin in mats at different heating intervals showed that devices A and B released bioallethrin uniformly during a period of 24 hr. Whereas in the case of device D, the release was higher during initial period of 4 hr and afterwards it was static at a low level upto 12 hr and there was a steady increase in the release upto 24 hr with more than 40% of bioallethrin available in the mat. There was a pronounced release of bioallethrin from the mat in the initial 1 hr period with the device C and the release remained static at a very low level for the remaining period of testing (24 hr). The amount of material released was found to be about only 30%. The device C, eventhough released bioallethrin at very low concentration, was found to be effective in the bioefficacy tests.

During the analysis of bioallethrin in mats with different heating devices using HPLC technique, it was observed that the concentration of bioallethrin in different mats was found to vary markedly eventhough each mat was expected to contain an active ingredient of 40 mg. Moreover, it was also noticed that the concentration of bioallethrin in the same mat was not uniformly distributed.

5.2.4. Effect of insect growth regulator, OMS 3031, on vector biology and its effect on non-target organisms:

Eventhough insecticidal treatments in the field are aimed at specific groups of organisms, effects are never limited merely to the target species. Any untoward effect of these chemicals on the natural regulators can cascade into a series of highly undesirable adverse effect on the whole environment resulting in resilience of vector population. In continuation with the studies on the effect of IGRs on vector biology and non-target organisms, an attempt was made to elucidate the effects of OMS 3031 on larvivorous fish, predatory insects, cyclopoid copepods, ostracods and nematode parasites which are important biological control agents in regulating mosquito population in nature.

Larvivorous fish:

OMS 3031 was found to be nonlethal to four species of larvivorous fish, *Poecilia reticulata, Gambusia af*

finis, Aplocheilus blochii and *Tilapia mossambica*, at 1.0 mg/l. The fish were found to be tolerant with higher LD_{50} values ranging from 2.21-3.097 mg/l. Absolute survival (100%) was observed in all the four

Table 5.4.

Bioefficacy of bioallethrin mats against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* using different heating devices.

Hours	A		B		C		D	
	% K.D	% M	% K.D.	% M	% K.D.	% M	% K.D.	% M
			Cx. quinquefasciatus					
1	86	19	97	14	90	4	70	8
2	98	10	98	28	82	6	81	6
3	99	25	97	32	74	7	86	4
4	100	21	98	45	69	4	92	3
5	96	16	97	59	81	21	97	6
6	98	15	98	63	84	20	100	4
7	86	0	93	93	80	28	98	21
8	82	19	90	91	82	53	97	14
9	75	33	95	96	86	45	97	6
10	61	9	94	45	78	86	90	12
Ctrl	6	3	4	5	7	4	5	0
			Ae. aegypti					
1	98	23	89	4	75	87	90	59
2	88	22	96	100	81	79	96	79
3	90	56	90	98	86	92	98	91
4	95	31	95	97	88	80	98	97
5	92	46	95	99	92	93	96	91
6	100	33	98	100	90	95	82	82
7	90	47	90	94	80	98	84	73
8	92	32	85	94	79	75	82	81
9	90	16	25	80	80	37	77	78
10	90	29	65	87	71	46	75	72
Ctrl	0	8	8	8	17	5	38	1
			An. stephensi					
1	97	92	42	74	62	62	97	7
2	99	85	94	60	71	70	95	61
3	98	79	92	54	68	56	92	60
4	100	78	94	79	83	90	98	38
5	100	78	96	79	96	98	100	22
6	98	69	95	82	98	96	100	33
7	99	81	94	79	79	91	98	60
8	90	87	86	80	65	77	99	73
9	86	72	82	77	54	77	93	70
10	76	61	ND	ND	68	90	90	76
Ctrl	2	6	4	4	3	3	2	2

KD - Knock down (%) ; M - Mortality; Ctrl - Control; ND - Not done ; A,B,C,D - Different heating devices

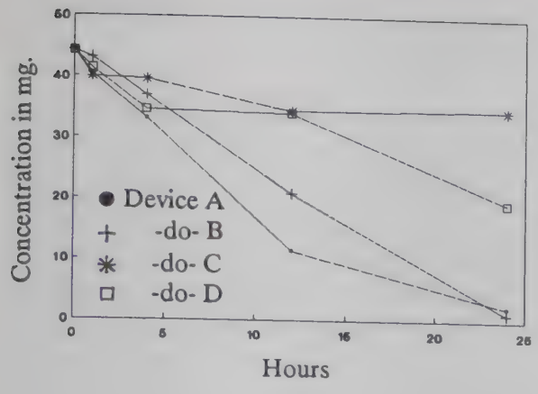


Fig. 5.7 Availability of bioallethrin in mats from different heating devices.

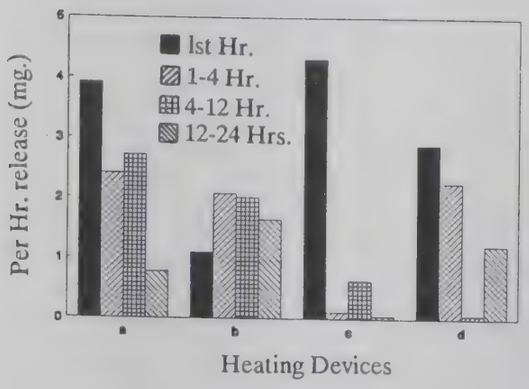


Fig. 5.8 Release rate of bioallethrin.

species at 1.0 mg/l for more than 8 days. Fish safety factor or suitability index computed for each fish species against the three vector mosquitoes showed maximum value for *P.reticulata* which indicated its higher tolerance than other species (Table 5.5). Slight decrease in swimming movements exhibited by the fish on treatment (at 1.0 mg/l) was restored when the exposure was withdrawn. No adverse effect was noticed in the reproduction of *G.affinis*.

Predatory insects:

The predatory insects, *Anisops bouveri* and *Diplonichus rusticus*, were not susceptible to OMS 3031 upto 1.0 mg/l, even after 48 hr of exposure. The predatory capacity of these insects was not affected by IGR treatment at the EI_{50} level of mosquito larvae.

Copepods and Ostracods:

Cyclopoid copepod *Mesocyclops leukarti*, a predator on mosquito larvae coexisting in the breeding places, showed lesser susceptibility to OMS 3031 with LC_{50} of 0.0774 mg/l, which was 7 and about 2 times higher than that obtained for two other IGRs, Dimilin and OMS 3013 respectively. Ostracods, the planktonic

Table 5.5.

Suitability Index/Fish safety factor (in ratio) of a fish species and a mosquito species.

Name of fish species	SI/FSF		
	Mosquito species		
	An.stephensi	*Ae.aegypti*	*Cx.quinquefasciatus*
P.reticulata	902.87	4936.79	7722.82
G.affinis	879.24	4807.58	7520.69
A.blochii	781.72	4274.46	6686.71
T.mossambica	647.52	3540.57	5538.65

organisms usually present in the breeding habitats, were not affected by the treatment of OMS 3031 even at a dose of 1.0 mg/l.

Parasitic nematodes:

Preparasitic nema of *Romanomermis iyengari*, a nematode parasite of mosquito, were less susceptible to this IGR when compared to mosquito larvae. But, significant ($P < 0.05$) mortality (17.14-38.53%) was noticed at concentrations which gave about 50% mortality in mosquito larvae. The infectivity of ppn on *Cx. quinquefasciatus* second instar larvae was not drastically affected by IGR treatment. However, the variations in infectivity were found to be insignificant. Postparasitic nematodes did not show any mortality at doses 0.0001-1.0 mg/l of OMS 3031. The treated post parasitic juveniles could moult to adult stage and were capable of surviving for more than 10 days at the concentration of 1.0 mg/l, which would give 100% mortality in mosquito larvae.

The effectiveness of IGRs in the control of vectors is usually assessed by their emergence inhibition rates alone, which necessitated frequent applications, thereby limiting its usage in control operations. IGRs, besides preventing adult emergence also have potential to suppress vector population even at sublethal levels by affecting the reproductive potential, hatchability, feeding and mating of adults that emerged from the immatures exposed to such dosage levels indicating that the frequent application is not essential. Further, negligible toxicity of this IGR towards non-target organisms makes it an ideal control agent for integrating in vector control programme. Hopefully, the future will see more compounds of this type with added advantages of safety, selectivity and specificity.

6. ECOLOGICAL STUDIES

6.1. STUDIES ON THE BIOLOGY AND BEHAVIOUR OF *ARMIGERES SUBALBATUS:*

Several aspects on the biology and ecology of *Armigeres subalbatus* in Pondicherry were discussed in the preceding annual report and in continuation, results of the investigations carried out on the habitat, physico-chemical characteristics therein, life budget analysis of immatures, surfacing behaviour of immatures, emergence rhythm, natural survival, insemination rate, oviposition behaviour & rhythm and susceptibility of larvae and adults to insecticides are presented here.

Various habitats supporting mosquito breeding were surveyed. Septic tanks were observed to be the only perennial breeding habitat of *Ar. subalbatus* in both urban and rural areas. The distribution of septic tanks in relation to households showed a proportion of 0.72 in urban which was significantly higher than that of rural area (0.33). Receptacles were found to serve as sporadic breeding habitat for this species. The positivity rate of septic tanks during the year ranged from 0.66% in July to 8.68% in February. The proportion of septic tanks supporting breeding was significantly higher in urban area (4.47% of 4367 surveyed) than in rural area (1.81% of 443 surveyed). Only a maximum of 0.29% of the receptacles were found to support *Ar. subalbatus* breeding.

Physico-chemical characteristics of water from selected septic tanks showed that the pH ranged within very narrow limits of 0.5 (8.0 - 8.5), a total absence of dissolved oxygen, BOD fluctuated between 10 and 190 mg/l, high values of total solids (18618 mg/l) and ammonia nitrogen (25 mg/l). The values of other chemical factors such as nitrate nitrogen (0.2 mg/l), salinity (198 mg/l), acidity (86.5 mg/l) and alkalinity (757.2 mg/l) were also estimated. Only ammonia nitrogen had significant relationship with immature density of *Ar. subalbatus*.

The relative density of immatures monitored in selected septic tanks at fortnightly interval showed a major peak in the month of March (139 per dip) and a minimum in May (30 per dip). The proportion of

first instar larvae was relatively higher in the month of February indicating a high recruitment.

Life budget analysis of immatures based on the samples collected from natural habitat during the months of February, June and October showed natural immature survival of 1.81, 5.71 and 23.75 percent respectively. Inspite of low temperature that prevailed in the month of February, which is expected to increase the survival of immatures, the survival was estimated to be the lowest and this could be attributed to the density factor. Highest immature survival recorded in the month of October reflected the prevailing favourable climatological factors such as temperature.

Observations on the surfacing behaviour of different immature stages showed that there was a gradual increase in the frequency of surfacing from first instar to pupa. Similarly, the time spent on the surface at every occasion and the total duration spent on the surface by the first instar larva was the least and that of pupa the maximum. The duration spent on the surface was not significantly different between the early instars (first and second) whereas it was significantly higher in the pupa when compared to fourth and third instar.

Emergence observed directly from pupae collected from field showed that it was continuous throughout the 24 hours. While there was a peak between 18.00 and 19.00 hrs. in males, no distinct peak was registered in females. The proportion of females (63.4%) emerged at night was significantly higher than that of males (36.6%). The results on hourly emergence determined through trap collections showed a peak between 16.00 and 17.00 hrs. following which there was a sharp decline for both sex. Also, the proportion emerging during day outnumbered those at night in case of both males and females. It was evident from these observations that adults that emerged at night preferred to stay within the septic tanks till day break.

Laboratory observations showed that in a male to female ratio of 1:1 over 50.0% insemination was achieved with 72 hrs. old adults. When the male to female ratio was increased to 2:1 and 3:1 similar results were obtained. The sex ratio when reversed resulted in a decline in the rate of insemination. It is inferred from the observations that mating could occur when both males and females were just 24 hours old. Nevertheless, with increase in age higher rates of insemination was attained.

The females preferred a solid object to lay the eggs. Pieces of brick partly immersed in water attracted more females than cotton. 88.8% of females laid eggs in the former while 11.2% on the latter when both were offered simultaneously. 24 hours observation made on the oviposition activity showed that a peak occurred between 16.00 and 18.00 hrs. The proportion of females ovipositing was high during the day (0.92) while it was very low (0.08) at night. Egg retention was found to be a natural phenomenon in *Ar. subalbatus*. There was a significant correlation between the proportion of females that retained eggs and age of the mosquito as well as between the number of eggs retained and the age.

Parity status of the resting population was ascertained for each month, based on which the daily survival rate was calculated. The proportion of parous females ranged between 0.25 and 0.50 in the urban area and daily survival rate ranged between 0.758 and 0.872. Highest survival rate was observed during February and lowest during June. The data on per man hour density was used to arrive at the finite rate of increase for different months and this ranged from 0.977 to 1.018, with values above 1 for the months of February, July, August, September, October and December. The survival rate in the rural area ranged between 0 and 1 and the finite rate of increase between 0.955 and 1.055. However, as the density level was low for most of the months, this was not comparable with the data obtained for the urban area. Life table was constructed for the months of February, June and October based on natural immature survival rates. When the finite rate of increase calculated was compared with the observed values it did not differ markedly.

Precipitin test for blood meal identification showed that high proportion fed on bovine and the Human Blood Index (HBI) was 0.24, revealing its zoophilic nature.

Field collected larvae were tested against selected insecticides, namely, fendona, fenthion and fenitrothion and the LC_{50} values obtained were 0.000006 mg/l; 0.00029 mg/l and 0.0017 mg/l respectively. The synthetic pyrethroid was found to be more effective than the other two. Adults were tested against DDT, malathion and fendona. Exposure to

diagnostic doses of DDT (4%) and malathion (5%) resulted in 100 per cent mortality of adults respectively in 5 hours and 15 minutes. The LD_{50} value of fendona was found to be 0.697 ug/cm^2, on standard exposure for 1 hour.

6.2. STUDIES ON PREDATOR-PREY INTERACTIONS:

Reports from both laboratory and field studies have suggested the use of the genus *Toxorhynchites* in the biological control of container and tree-hole breeding mosquitoes. But the quantitative information on population interaction of this predator and its prey species and factors influencing such population oscillation is meagre. To study such interactions laboratory experiments on cage population of *Ae. aegypti* were carried out. The cages consisted of the usual type used for laboratory colonization of mosquitoes, measuring 1 m^3.

In first experiment, first instar of *Ae. aegypti* and fourth instar of *Tx. splendens* at the ratio of 50:1 were introduced in a colony cage. No adults were released and the oviposition trays provided did not have any floating materials. The density of prey and predator was monitored for 18 weeks. The oscillation in prey-predator population is depicted in Fig.6.1a.

In the second experiment prey and predator ratio was same but the oviposition trays were provided with floating materials and the populations were monitored for 29 weeks and the oscillation is depicted in Fig.6.1b. Though the experiments in both cage-1 and cage-2 were started with same number of prey and predator larvae, in cage-2 the interaction between prey and predator population lasted for 29 weeks, 11 weeks more than that of cage-1. The prolongation in the population interaction between prey and predator in this case is perhaps the result of structural complexity in the oviposition trays which facilitated recruitment.

In third set of experiment 500 first instar *Ae. aegypti* and 10 fourth instar *Tx. splendens* were introduced along with 25 pairs of *Tx. splendens* adults. At the end of the 1st week another 25 pairs of adults were introduced. Because of more number of predator during initial period of the experiment, the chance of *Ae. aegypti* population to establish was negligible. The prey population dropped to zero within 2 weeks and no prey larvae were available for predator till 8th week. This starved the predator and its number reduced to zero in week 6 and there after no *Tx. splendens* larvae were found in any of the container till the end of the experiment. But from week 9 onwards, there was a gradual increase in *Ae. aegypti* larval population and attained the maximum density of about 2,300 in week 15 (Fig.6.1c). In this the popula

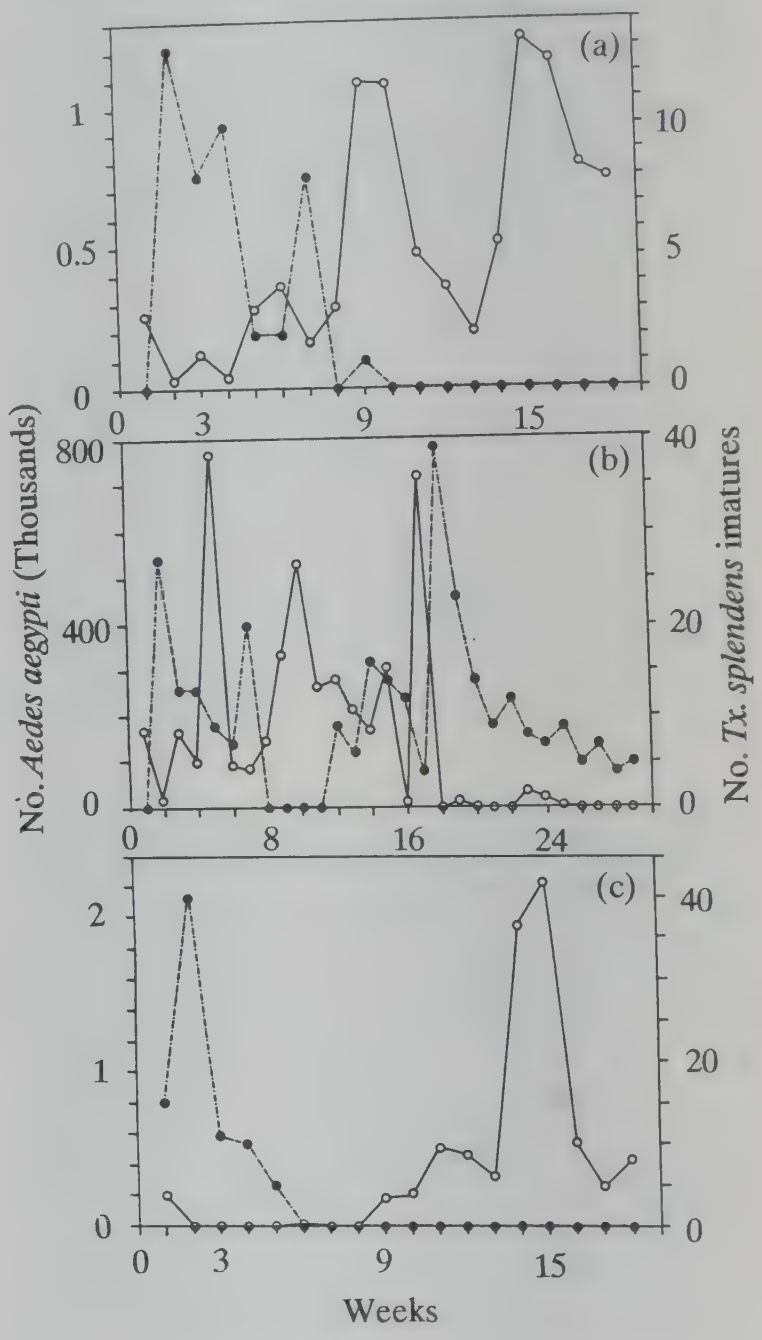


Fig. 6.1 Relationship between prey (○ *Ae. aegypti*) and predator (● *Tx. splendens*) larval poulation in simulated container habitats. Expt. I - No adults were released and no structures provided (a); Expt. II - No adults were released but structures provided (b) and Expt. III - Adults were released but no structure provided (c).

tion of *Tx. splendens* and *Ae. aegypti* cycled in a way similar to the cage 1 populations. In both the cases observations were made upto 18 weeks. However, the differences between these two results clearly demonstrated that the initial population density of prey and predator and the complexity of the environment play crucial role in establishing stable interaction between prey and predator in the natural condition.

6.3. STUDIES ON SANDFLIES:

6.3.1. **Colonization of Sandfly:**

A cyclic colony of *P. papatasi* was successfully established from wild caught females. The propagation of the colony was found to be affected by various factors. When the immatures were fed on animal liver powder, infestation of the food material with fungi was found to be the major problem and it affected the immature survival adversely. Though the fungi did not infest the larvae, they were seen to obstruct their movement and thereby their feeding, resulting in heavy mortality. To prevent the fungal growth, the food material was subjected to aging before use. Since this method was found to be laborious and time consuming, the food material was sterilized and used as larval food. Fungal growth was observed even in sterilized liver food. However, when the faecal pellets of rabbit was sterilized, powdered and mixed with white clay and used as larval food, the problem of fungal growth was minimised to the tolerable level in subsequent generations.

Once the females started laying eggs regularly, no more field collected adults were introduced. Through generations the progenies adapted themselves for mating, feeding and oviposition under laboratory conditions. In this process a stable, cyclic colony was established. The colony is in F^{25} generation.

When the productivity of the colony of *P. papatasi* was studied with 500 females, a total of 19,019 eggs was obtained during next five consecutive generations. A total of 2,384 eggs were obtained from 500 females and from which 714 females and 685 males emerged. From subsequent generation onwards the number of adults produced increased reaching a maximum level in the fifth generation.

6.3.2. **Biology of sand flies:**

Immatures:

Preliminary studies on the larval biology showed the occurrence of cannibalism among the immatures of *P. papatasi*. Hence a detailed investigation was carried out on this aspect by culturing known number of immatures in containers having a surface area of 7.25 cm^2. For this purpose four sets of experiments were made. In the first set the larvae were fed on a mixture of white clay and rabbit faecal pellets. In the second set the larvae were subjected for total starvation. In the third set they were fed on at the end of experiment and in the fourth set the larvae were given food material once in the beginning. Observations were made on the cannibalistic behaviour. The larvae found dead without any injury was accounted for natural mortality and the larvae found missing were considered to be consumed by other larvae. To confirm cannibalistic behaviour, gut contents were also analysed.

Cannibalism was found to occur in all the sets of experiments. In the first set, it was 3.2 ± 1.6 whereas in the second and third, it was 90.2 ± 4.9 and 83.0 ± 6.5 respectively. In the fourth set a mean of 86.7 ± 2.9 larvae were consumed. In the first set the pupation and emergence obtained were 87.2 ± 1.7 and 85.2 ± 1.2 respectively. This shows that in the presence of adequate food the larvae grew, pupated and emerged to adult. In the second set cannibalism was found to be maximum due to starvation and none of the larvae pupated.

Occurrence of cannibalism was also evident from the remnants of larval parts such as head capsules and caudal bristles in the rearing containers which could be easily differentiated from that of exuviae. In the exuviae of the moulted larvae the cuticle of head capsule and caudal bristles were transparent and intact in the remnants. The analysis of gut contents also showed the presence of larval parts in their guts, confirming the cannibalistic behaviour.

Immature duration and survival:

Studies were carried out to find out immature duration and immature survival under simulated field conditions for different seasons. The immature duration was 31, 39 and 43 days in summer, rainy and winter seasons respectively. The immature survival ob-

tained for the corresponding periods was 0.232, 0.579 and 0.604 respectively.

Adult:

Duration of gonotrophic cycle:

The duration of gonotrophic cycle was studied under ambient conditions. The duration of first and second gonotrophic cycle was 8-5, 9-6, and 11-8 days respectively in summer, rainy and winter seasons. Most of the females died after completing first gonotrophic cycle and the duration of second was calculated from those few females which survived until the second oviposition. The duration of subsequent gonotrophic cycle viz., third, fourth, fifth and so on was considered similar to that of the second gonotrophic cycle. This was based on the assumption that most of the females obtain single blood meal per gonotrophic cycle, all females complete each cycle in same number of days and repeat the cycle with same probability of survival as long as they live as was observed in mosquitoes.

Fecundity, sex ratio and longevity:

Observations were carried out on the fecundity, sex ratio and longevity using laboratory reared females. The fecundity observed was 56.2 $\pm$ 5.46 eggs per female in summer, rainy and winter seasons. The male to female sex ratio was 1:1 in all seasons. The longevity was found to be 27 days for all seasons.

6.3.3. **Population dynamics:**

A life and fecundity table for the population of *P. papatasi* was constructed for different seasons based on immature duration, immature survival, adult survival, duration of gonotrophic cycle, fecundity, sex ratio and longevity.

The net reproductive rate (R_o) was 6.6160 in summer (June), 18.9096 in monsoon (October) and 14.5290 in winter (February) season. The mean generation time (T) i.e., the duration from egg laying to emergence of adult was found to be low in summer and high in winter. The mean generation time was 44.39 days in summer, 53.79 days in monsoon and 59.11 days in winter.

Innate capacity to increase:

The infinitesimal rate of increase or innate capacity to increase (r_m) calculated for laboratory population was 0.0425 in summer, 0.0546 in monsoon and 0.0452 in winter season.

Finite rate of natural increase:

The finite rate of increase (λ) derived from life and fecundity table data was 1.04348 in summer, 1.05617 in monsoon and 1.04632 in winter. The data shows that monsoon is more favourable for the multiplication of *P. papatasi* population.

6.4. STUDIES ON MUSCOID FLIES:

Distribution and abundance of Hymenopteran pupal parasitoids of *Musca domestica* with their natural parasitism are being monitored at weekly intervals in fixed stations viz., dairy and poultry farms situated in and around Pondicherry. From the field collected puparia of Muscoid flies four parasitoids viz., *Splangia cameroni, Splangia nigroaenea, Dirhinus himalayanus* and *Pachycrepoideus vindemmiae* were recorded.

6.4.1. **Host parasitoid interaction and population dynamics:**

The host parasitoid interaction and population dynamics of *Pachecrepoideus vindemmiae* and *Dirhinus himalayanus* were studied in the laboratory. Ten freshly emerged female parasitoids from a single cohort were isolated and each was paired with a male and maintained on 50% honey. Daily 20 fresh *Musca domestica* pupae were exposed to them till the death of the female parasitoid. The exposed puparia were kept for emergence of fly/parasitoid. The net reproductive rate (R_o) calculated for these two parasitoids was 5.89 and 28.3 respectively.

Studies were carried out on the adult survival, fecundity and host destruction activity of *D. himalayanus* at different ranges of temperature (24, 27, 30, 33 and 36^{o}C). A declining trend in the survival was observed with the increase in temperature. Tempera-

ture also appears to influence the sex ratio of the progeny. Male to female sex ratio of parasitoids at 27, 30, 33 and 36°C was 1 : 2.2, 1 : 2.3, 1 : 6.2 and 1 : 29.6 respectively.

When virgin females of *D. himalayanus*, maintained on 50% honey were exposed to puparia of *M. domestica*, they produced only male progeny. The average number of progeny produced by a female was observed to be 10.5±3.38. This confirms the existence of parthenogenesis.

Laboratory studies on the influence of host density on adult survival, host destruction activity, reproduction, competitive parasitism, super parasitism, multiple parasitism and parasitoid behavior of *D. himalayanus* and *P. vindemmiae* are in progress.

6.4.2. Fly control by inundative releases:

Dirhinus himalayanus, a chalcidid fly-pupal parasitoid was mass cultured in the laboratory and inundative releases were carried out at weekly intervals. About 600 parasitoids were introduced in a dairy farm situated in Natesan Nagar, located 1 km. away from Pondicherry. Data on fly density as well as parasitoid density were collected prior to release. Post release data are being collected for analysis.

7. OTHER STUDIES

7.1. PROJECT FOR THE PREPARATION OF A MASTER PLAN FOR MOSQUITO CONTROL IN COCHIN CORPORATION AREA:

The Mayor of Cochin Corporation held discussions with the Director General of ICMR, Director and Asst. Director of VCRC, Pondicherry, regarding the mosquito problem of Cochin Corporation. Subsequently, based on the invitation from the Corporation for assisting them in the preparation of a Master Plan for the control of mosquitoes, a field unit was established at Mattancherry, Cochin during the month of June 1990. According to the memorandum of understanding signed by the Corporation and VCRC, the Corporation has provided a fully furnished accommodation with telephone facilities for laboratory/office, a vehicle, and the services of a draftsman, eight mazdoors and one driver for day to day field activities. The expenditure incurred on fuel is also being met by the Corporation. The initial duration of the project agreed upon is for 3 years from June, 1990. In return, the VCRC has to submit interim reports every year followed by a Master Plan when the studies are completed. Periodic recommendations will also be made by the VCRC for implementation for the control of mosquitoes by the Corporation. It was mutually agreed that the role of VCRC will be exclusively on an advisory capacity and the day to day mosquito control will remain with the Corporation.

Study area:

The city of Cochin, known as the Queen of the Arabian sea is surrounded by greenish outskirts in the east, Arabian sea in the west and extensive backwaters with bluish sea-mouth amidst. The Corporation of Cochin was constituted on 1st November 1967 by amalgamating 3 former Municipalities viz. Ernakulam, Mattancherry and Fortcochin and four panchayats viz. Vyttila, Palluruthy, Vennala and Edappaly, and a few Islands of Cochin. The total area of Cochin Corporation is 94.88 sq.kms, with 87,566 house holds and population of 5,13,249 as per 1981 census. The city is divided into two parts by back waters. The Corporation is broadly classified into East Cochin Zone and West Cochin Zone.

Present Organisational structure for mosquito control:

Cochin Corporation has a Health Dept. functioning directly under the Commissioner. This Dept. is headed by a Health Officer and consists of one Lady Medical Officer, two Assistant Health Officers, 21 Health Inspectors and 80 Junior Health Inspectors, 305 Anti-mosquito Workers. This Corporation is unique in having women for spray operations (Plate VI, A).

For the operational convenience the entire area is divided into twenty two circles and each circle is

under the control of one health inspector. Each circle is further divided into 50 divisions. There are 27 divisions in the East Zone and 23 divisions in the West. Each zone is under the direct supervision of one Assistant Health Officer.

National Filariasis Control Programme (NFCP) is also carrying out antilarval measures in selected pockets of corporation (12-18 divisions of Mattancherry and division 38 of Ernakulam) which is headed by one Senior Biologist with supporting staff of one Asst. Entomologist, three Filarial Inspectors, one Health Inspector, one Laboratory Technician, eleven Field Assistants, two Insect Collectors, one Maisthry, forty two field workers and two drivers besides the administrative staff.

Several studies have been initiated to understand the species composition of mosquitoes in order to recommend suitable control measures.

Man-biting collections:

Whole night man-biting collections were carried out at Cochin and adjacent islands to identify the man biting mosquitoes of this area. Since May 1990, a total of ten biting collections have been made both indoor and outdoors. Among these collections, two were carried out in Cochin mainland and the rest in Vypeen island.

A total of 17 species was obtained from biting collections. Among these, 8 species belong to the genus *Culex*, 3 belong to *Anopheles*, 3 *Mansonia* and 1 *Armigeres*. In the island situation, *Cx. sitiens* was found to be the predominant man biting mosquito followed by *An. subpictus* and in the main land *Cx. quinquefasciatus* was the dominant mosquito. Further investigations on aspects such as seasonal variation and abundance are in progress.

Indoor resting collections:

Indoor resting collections were carried out only at Vypeen island. The resting collections yielded seven species of mosquitoes consisting of 3 genera viz., *Aedes, Anopheles* and *Culex*. Among all the species *Cx. sitiens* was the predominant species followed by *An. subpictus.*

Major breeding habitats:

Towards the preparation of Master Plan, surveys have been completed in circle 1, 2 and 3 to identify the major mosquitogenic habitats. The following breeding habitats have been identified which cause major concern:

1. Drains and Canals carrying sewage and sullage.
2. Defective septic tanks (Plate VI, B)
3. Wells
4. Cement tanks
5. Water meter chamber
6. Ponds
7. Miscellaneous habitats like empty containers, grinding stones, flower pots, tyres etc.

The type of habitats and their surface area estimated for different circles are given in table 7.1.

Table 7.1.

Type and surface area of different breeding habitats of circle 1, 2 and 3 of Cochin Corporation.

Circle No.	Type of breeding habitats	Length in mts./No.	Surface in m^2
1	U drain	4,150	7,163
	Septic tanks	2,344	7,032
	Wells	336	336
	Cement tanks	65	3,570
2	Canal	1,400	21,000
	U Drain	61,641	9,729
	Septic tanks	852	2,456
	Wells	256	256
	Cement tanks	57	114
3	U Drain	12,950	10,236
	Septic tanks	986	2,958
	Wells	231	231
	Cement tanks	83	78

The problems and recommendations with regard to circle 1, 2 and 3 have been submitted to the Corporation for initiating remedial measures.

7.2. REARING AND COLONIZATION:

7.2.1. **Arthropod colonies:**

Cyclic colonies of the following insects are being maintained:

Mosquitoes:

Culex quinquefasciatus, Culex tritaeniorhynchus, Culex sitiens, Culex (Lutzia) fuscanus, Anopheles stephensi, Anopheles culicifacies, Armigeres subalbatus, Aedes aegypti, Aedes albopictus, Toxorhynchites splendens and *Mansonia annulifera.*

Housefly:

Musca domestica.

Cockroach:

Periplaneta americana.

Parasitic wasps:

Pachycrepoideus vindemmiae, Dirhinus himalayanus, Spalangia sp. and *Tetrastichus hagenowii.*

7.2.2. **Production and supply of insects:**

The details on the mass production of various insects are given in Table 7.2. The materials supplied to different sections in the Institute and other Institutes for different bioassay studies and other purposes are shown in Table 7.3.

Table 7.2.

Production of different species of insects

Name of the species	No of ootheca	No of pupae
1. *Cx. quinquefasciatus*	-	64,80,000
2. *Cx. sitiens*	-	4,32,000
3. *Cx. tritaeniorhynchus*	-	3,60,000
4. *Ae. aegypti*	-	10,80,000
5. *Ae. albopictus*	-	36,000
6. *Ar. subalbatus*	-	2,88,000
7. *An. stephensi*	-	36,00,000
8. *An. culicifacies*	-	3,60,000
9. *T. splendens*	-	1,08,000
10. *M. annulifera*	-	36,000
11. *M. domestica*	-	1,50,000
12. *P. americana*	15,000	

Table 7.3.

Details on the materials supplied

Name of species	Larvae	Pupae	Adults	Total
1. *Cx. quinquefasciatus*	90,12,250	2,47,700	1,11,000	93,70,950
2. *Cx. sitiens*	46,000	35,000	10,000	91,000
3. *Cx. tritaeniorhynchus*	74,000	53,000	20,000	1,44,000
4. *Ae. aegypti*	12,98,600	75,500	27,900	14,02,000
5. *Ae. albopictus*	4,000	2,000	---	6,000
6. *Ar. subalbatus*	75,000	55,000	10,000	1,40,000
7. *An. stephensi*	14,18,600	76,000	42,700	15,37,900
8. *An. culicifacies*	28,000	10,500	---	38,500
9. *T. splendens*	4,800	9,600	100	14,500
10. *M. annulifera*	1,100	---	---	1,100
11. *M. domestica*	1,000	---	200	1,200
12. *P. americana*	---	---	750	750
13. *P. papatasi*	190	---	200	390

7.2.3. **Standardization of marking techniques of mosquito larvae:**

To study the dispersal behaviour of any vector population under natural condition, mark release recapture technique could be used. Hence a study was made to stain some of the mosquito larvae using Giemsa, Methylene blue and Eosin dyes in different concentrations. Aqueous solution of these dyes were prepared by dissolving in distilled water. Fourth instar larvae of *Culex quinquefasciatus, Cx. tritaeniorhynchus, Cx. sitiens, Aedes aegypti, Armigeres subalbatus, Anopheles stephensi, An. culicifacies* and *Mansonia annulifera* were exposed to 0.01%, 0.02% and 0.04% aqueous solution of Giemsa, Methylene blue and Eosin for 12 hours. Later the larvae were filtered out and transferred to tap water and reared to adult stage by feeding with standard larval diet.

Daily about 5 adults were dissected and the visceral organs were examined under microscope to find out the persistence of stains. *Cx.tritaeniorhnynchus* was found to retain Giemsa for about 8 days and Methylene blue for 10 days. *Ar. subalbatus* was seen to have the stain Eosin for 3 days while the rest for 2 days.

7.2.4 **Experimental Animals:**

The central animal house provides a regular and adequate supply of laboratory animals of desired age, sex and weight to various departments for their research work. The total number and different types of animals as on 20.12.90 are given below:

Sl.No	Type of animal	Total number	Experiments for which they are used
1.	Albino mice	124	Toxicology experiments
2.	BALB/c mice	100	Raising monoclonal antibodies
3.	Albino rats	119	Toxicology experiments
4.	Guinea pigs	23	Toxicology experiments
5.	Rabbits	31	Toxicology and serology experiments and also for feeding mosquito colonies.
6.	Fowls	26	Feeding mosquito colonies and maintenance of *Plasmodium gallinaceum.*

7.3. VECTOR GENETICS

7.3.1. *Culex quinquefasciatus:*

Insecticide Resistance:

Development and reversion of Malathion/Fenthion resistance in *Cx. quinquefasciatus* population in Pondicherry was reported earlier (Annual Report 1989).

Malathion and fenthion resistant genes are semi dominant in character, using the discriminating dosages, the field population were characterised into the three genotypes RR, Rr and rr with respect to both the insecticides.

During 1989 and 1990, malathion resistant homozygotes have become predominant and homozygous susceptible individuals were almost completely eliminated.

With fenthion, the percentage of homozygous resistance gradually came down from 95% to 88% and the homozygous susceptibles have increased from 5% to 12%.

Similar tests with laboratory *Cx. quinquefasciatus* showed that the percentage of malathion resistance remained almost stable whereas in fenthion susceptible homozygotes have become predominant.

Biology and genetics of field susceptible Cx. quinquefasciatus to Wuchereria bancrofti:

In a total of 3,182 *Cx. quinquefasciatus* dissected, the infection and the infectivity rate were 10.49% and 1.10% respectively. The infective mosquitoes had either progressive stages with infective larvae or only infective stage. Majority of the infective mosquitoes had one or two infective larvae while in others it ranged between 3-8. In addition, most of them had two dilatations indicating that these mosquitoes had 2 blood meals, the first being from a microfilaraemia carrier and the two ovipositions, sufficient for the filarial larvae to reach the infective stage.

Two generation test crosses of the male progeny of infective females with three different sex linked recessive eye color mutants have shown a specific genetic pattern and the evidence for chromosomal inversions. Very few mosquitoes had shown delayed egg laying and failure of the first stage filarial larvae

to develop further at the end of the normal incubation period.

Mutants:

A reddish eye color mutant has been isolated from the natural population of *Cx. quinquefasciatus*. Genetic analysis shows that this mutant is a sex linked recessive, not an allele of apricot eye or pinkish eye and 17% crossover units from the male determining locus.

7.3.2. *Anopheles stephensi:*

Two distinct Y chromosomes (Y_1 and Y_2) have been identified in the field population of *An. stephensi*. Y_2 type is predominant in the malarial endemic area. The presence of two types of Y chromosomes suggests and supports the hypothesis that Y_2 has been derived by a terminal detection.

A brown eye color mutant has been isolated from the natural population of *An. stephensi*. In the larval stages the eye color is hardly recognisable but the expression is good at the late pupal and adult stages. The mutant is an autosomal recessive.

7.3.3. *Anopheles subpictus:*

Relative susceptibility of the nematode *Romanomermis iyengari* in a natural larval population of *An. subpictus* complex was studied. *An. subpictus* complex consists of four morphological types, two fresh water (A,C) one salt water (B) and one breeds in salt and fresh water (D). The nematode *R. iyengari* is completely absent in salt water breeding *subpictus*. In fresh water all the three types A, C and D are positive but the susceptibility of A is higher as compared to C and D.

7.4. MOSQUITO BLOOD MEAL SOURCE IDENTIFICATION:

The standard gel diffusion technique is employed routinely for blood meal source identification. The antisera for this purpose are raised in rabbits. Between January and November, 1990, a total of 3,299 filter paper blood samples have been tested. The results are shown in Table 7.4. Most anophelines both from Jeypore and Malkangiri areas were zoophilic, excepting *An. fluviatilis* which recorded a high HBI of 0.83 in Malkangiri locality. Of the *Mansonioides* the HBI of *M. annulifera* was the highest (0.98).

IV. MISCELLANEOUS

1. EDUCATIONAL PROGRAMME

Admissions to the M.Sc. (Medical Entomology) course of the Centre which is affiliated to Pondicherry University are made after an All India written entrance test (Plate VII, A). The course has been recognised by the WHO/TDR programme as the best run course in Medical Entomology and encompasses a thorough training in all theoretical and practical aspects, including extensive field studies (Plate VII, B).

Eight students were admitted to the M.Sc. Medical Entomology programme during the 1990-91 academic session, out of whom, 6 are fresh graduates and 2 are inservice candidates.

The Third batch of 10 M.Sc. students graduated in May, 1990. The summaries of the dissertation submitted by them are given below:

1. *Evaluation of controlled release formulations of Mosquito larvicides against Culex quinquefasciatus Say 1823.*

Controlled release formulations (pellets) of two organophosphorus larvicides viz., fenthion and temephos using chemically modified biodegradable

Table 7.4

Specieswise analysis of mosquito blood meal source

Species	Jeypore					Malkangiri				
	Human	Bovine	Avian	No Reaction	Total	Human	Bovine	Avian	No Reaction	Total
An. fluviatilis	15	76	0	1	92	297	60	0	1	358
An. culicifacies	1	662	0	1	664	11	900	0	8	919
An. annularis	0	56	0	0	56	0	176	0	0	176
An. aconitus	0	2	0	0	2	1	14	0	0	15
An. jeyporiensis	0	113	0	0	113	1	15	0	0	16
An. maculatus	0	25	0	0	25	1	16	0	0	17
An. philippinensis						0	1	0	0	1
An. varuna						9	233	0	1	243
Total	16	934	0	2	952	320	1415	0	10	1745
	Shertallai									
Mn. annulifera	296	7	0	0	303					
Mn. uniformis	51	156	0	0	207					
Mn. indiana	5	0	0	0	5					
Total	352	163	0	0	515					
	Pondicherry									
Ar. subalbatus	20	56	0	2	78					
Cx. tritaeniorhynchus	5	4	0	0	9					
Total	25	60	0	2	87					

material have been developed due to improvement of application technology in the use of mosquito larvicides. One of the formulations of fenthion and temephos (F_2 and T_2) was found to be intact during a period of 12 weeks. It has been found that the application rate of 1 pellet/100 lit. could prevent breeding of *Cx. quinquefasciatus* for more than 5 weeks with the concentration of the larvicide ranging between 0.04 and 1.4 mg/l in the case of fenthion and 0.04 and 0.7mg/l in the case of temephos. The pellets were found to be stable throughout the study period. This type of controlled release formulations can play an useful role in larval control operations because they minimize man-power requirement, consumption of insecticide, frequency of application, pollution to the aquatic environment and non-involvement of spray equipment as in the conventional spraying.

2. *The microbial flora in the gut of Culex quinquefasciatus Say (1823) larvae breeding in cesspits.*

The number of different types of microorganisms in the gut of *Culex quinquefasciatus* larvae vary considerably, from one site of collection to another and larval gut in general contains enormous number of bacteria, a few fungi and negligible number of actinomycetes. Fifteen bacterial, 6 fungal and 4 actinomycete genera were found. Among bacteria,

Bacillus sp., *Staphylococcus* sp. and *Pseudomonas* sp. were encountered more frequently than the remaining 12 genera. Genus *Aspergillus* among the fungi and *Streptomyces* sp. among the actinomycetes were frequently encountered. *Escherichia* and *Proteus* among the bacteria, *Aspergillus* among fungi and *Streptomyces* among actinomycetes were the most abundant genera found in the *Cx. quinquefasciatus* larval guts.

Isolates belonging to the genera *Bacillus, Pseudomonas, Shigella* and *Staphylococcus* caused 100% mortality during the early instar of larval development. None of the fungal isolates effected 100% mortality while *Nocardiopsis* sp. among actinomycetes gave 100% mortality during the first instar. Some of the isolates belonging to the genera *Escherichia, Bacillus* and *Flavobacterium* inhibited emergence of adults significantly. Among fungal isolates *Aspergillus* and *Alternaria* considerably inhibited the emergence and among actinomycetes, 2 isolates of *Streptomyces* and one isolate of *Nocardia* allowed negligible percent emergence.

3. *Studies on Malaria persistence in Rameswaram island with special reference to the period from 1985-1988.*

It is quite evident that the people in Rameswaram are well aware of malaria problem and their co-operation with the surveillance worker is observed to be good. There were some constraints such as inability to cope with the increasing malaria cases for contact smear collection, difficulties in completing radical treatment particularly among the fishermen who usually attend fishing after partial relief from malaria attack following presumptive/first day radical treatment etc.

It was observed that during the period 1985-87 malaria problem in Rameswaram island was tackled efficiently with the infrastructure and facilities provided in MPO, inspite of certain deficiencies and constraints. However, these achievements could not be maintained in 1988 and 1989. This might be due to the reason that full staff complement as per MPHW scheme was not provided initially.

The current organizational pattern following the implementation of MPHW scheme and the induction of new categories like Block Health Supervisors and Community Health Nurses at the PHC level with responsibility and authority over field staff (resulting in partial delinking of Medical Officers from the preventive aspects) (Tamil Nadu Government Order No.355, 1989) appears to be a good sign of improvement in the development of health infrastructure.

4. *Dengue fever/Dengue hemorrhagic fever (D.F/D.H.F) and the principal vector, Aedes aegypti (L), in the World - A Review.*

The vital factor for effective dengue abatement is a system for rapid transmission of information and cooperation among affected countries, an activity in which WHO has been instrumental since the recognition of DHF.

A long-term *Ae. aegypti* control combined with public cooperation in reducing household breeding sites seems at the moment the only means of containment of the disease. In Singapore when *Aedes* house index was 1%, transmission among susceptible people was maintained. This shows that, unlike Yellow fever, the *Aedes* house index will have to be brought down to 0, to prevent transmission. An impossible task as there are and will always be alternative breeding sites. Thus an intensive surveillance and long-term control efforts with the help of the community can be the only key to combating dengue.

5. *A review of Leishmaniasis in India.*

From the earliest Jessore epidemic upto the 1930s leishmaniasis was rampant in India. In the 1940s and 1950s the trend reduced due to setting up of treatment centers, increase in immunity and DDT spray under antimalaria programmes. From the 1960s to early 1970s the disease was almost a rarity but an outbreak occurred again in Bihar in 1977 and in Bengal in 1980. The trend today is that cases are increasing and an epidemic is expected in 1992. Epidemics occurred in 5 year waves with inter epidemic periods varying upto 15 years. The disease principally affects children and young adults. Kala-azar is rampant in tribal, rural and suburban areas in the eastern parts of the country. The vector sandflies are still very susceptible to DDT and dieldrin. Only one case of resistance has been reported against *P. papatasi* in Bihar.

6. *Urban malaria situation in Madras city - A Review.*

The following suggestions, if implemented could improve the control of malaria in the city. They are: To tackle the problem of breeding in overhead tanks of the houses, a ladder in order to have better access to the tank should be insisted upon in the 25 divisions with high malaria incidence; The various provisions available in the Tamil Nadu Public Health Act and the M.C.M.C. Act of the corporation should be strictly enforced so as to achieve maximum efficiency in mosquito control. Health education should be given on the matter of concern, to the public, using the mass media like television and radio. Drinking water wells should be hermetically sealed in order to prevent vector breeding. For extraction of water, suction pump should be provided over the well top or installation of pumping set be provided by the side of the well.

7. *Some studies on Bacillus thuringiensis H-14 and Bacillus sphaericus H 5a 5b toxic to mosquito larvae.*

B.thuringiensis H-14 and *B.sphaericus* strains grown in NYSM produced higher biomass and sporulated better. The indigenous isolates of *B.thuringiensis* H-14 and *B.sphaericus* isolated at VCRC were found to be more potent in all the respects than the standard strains. *Cx.quinquefasciatus* larvae were found to be 8-15 times more susceptible than *An.stephensi* larvae to *B.thuringiensis* H-14 strains and 4-6 times to *B.sphaericus* strains. The percentage mortality produced by the combination of *B.thuringiensis* H-14 and *B.sphaericus* was always found to be lower than when the two bacteria were tested individually.

8. *Studies on Biochemical changes in the parasitism of mosquito larvae by Lagenidium sp.*

The present investigation has revealed many unique features with respect to biochemical changes in *Cx.quinquefasciatus* larvae following infection, with the VCRC isolate of the fungus, *Lagenidium*.

Production of hydrolytic enzymes of high specific activity by the germinating zoospores may primarily aid penetration of insect cuticle and probably virulence is more likely to be correlated *in vitro* with enzyme production by germinating zoospores. Once entered, the fungal isolate appeared to overcome susceptible hosts primarily by utilization of the available nutrients in the haemocoel. Enzymes particularly proteases, presumably are important also for rapid fungal growth after invading the host. The acid phosphatase and glucosidase secreted by the fungus could probably play a role in the latter part of pathogenesis by way of generating toxic free phenols out of phenyl glucosides and phenyl phosphates present in the insect hemolymph and cuticle.

9. *Flora and fauna of mosquito breeding habitats - A Review.*

Mosquito larval habitats, by virtue of their complexities and diversities, provide home for a wide variety of elements of the biotic community. Parasites, predators, pathogens, commensals, symbionts, etc., that co-habit larval habitats of mosquitoes, correspondingly exercise their influence, in conjuncture with the abiotic factors and thereby prohibit/inhibit/restrict the breeding potentials of the mosquitoes. Some of the notable interactions are as follows:

Algae serve as excellent food source to mosquito larvae. eg., some members of Conjugales, Bacillariophyceae and Chlorococcales. Presence of certain algae favor abundant or preferred breeding of *An.varuna, An.stephensi, An.annulipes* and *An.culicifacies*. Some algae protect larvae from predation by fish. eg., *Chaetomorpha* and *Enteromorpha* in backwaters. Blue green algae and a few green algae inhibit growth/development or cause mortality of larvae. eg., *Oscillatoria, Anabaena, Scenedesmus* and *Chlorella*.

Epibionts like *Vorticella* infect larvae and restrict their movement or kill them (*An.annulipes* and *Ae.albopictus*). Protozoans, *Cyclops, Daphnia* and hydrachnid mites attack and kill anopheline larvae.

Macrophytes provide necessary protection and shade to mosquito larvae. eg., i) flexuous vegetation in reservoirs (*An.quadrimaculatus*) and ii) *Pistia* and *Eichhornia* (*An.funestus* and *Cx.vishnui*). They provide oxygen. eg., *Eichhornia, Pistia* and *Salvinia* in ponds (Mansonioides) and favor oviposition (*An.hermsi*). They promote breeding by protecting larvae from wave action. eg., *Cyanodon dactylon* in back waters (*An.subpictus*). Some macrophytes interfere with respiration or cause death due to asphyxiation. eg., *Azolla, Lemna* and *Wolfia* (*An.sinensis* and *An.annularis*). The bladderwort, *Utricularia* kills larvae by entrapping. A few macrophytes are actively poisonous. eg., *Brasenia* in marshes, lagoons and ponds (*An.quadrimaculatus*). Thick growth of mac-

rophytes and floating vegetation act as barriers to oviposition and thus inhibit breeding of *An.albimanus, An.culicifacies, An.fluviatilis, An.minimus* and *An.vagus*.

Most of the macrofauna predate upon the immature stages. eg., i) *Toxorhynchites* sp. in tree holes, rock pools, leaf axles, containers etc., ii) Notonectids in ponds, rice fields and back waters (*An.sundaicus, An.subpictus* and *Cx.vishnui*), iii) Ephydrid and Dolichopodid flies (Culicines and Anophelines), iv) *Laccotrephes griseus (Culex* and *Aedes*) and v) *Lacconectus (Ae.albopictus*).

10. *Selection of vector mosquitoes resistant to Permethrin*.

The study was carried out to find the use of synthetic pyrethroids in controlling urban malaria vector, *Anopheles stephensi* with special reference to the problem of resistance development. The parent strain of the test species, *An.stephensi* was selected with permethrin at LD_{90} level upto five generations. The present strain was quite susceptible to DDT (4%), malathion (5%) with the respective percentage mortalities of 92.5 and 86.25 at 1 hr exposure and pyrethroids (permethrin, cypermethrin, deltamethrin and alphamethrin) with LD_{50} values (mg/cm^2) of permethrin (0.68), cypermethrin (0.68), deltamethrin (0.47) and alphamethrin (1.0).

It has been found that the selection by permethrin has resulted in the development of resistance in F_5 generation to a 13-fold increase to permethrin and cross resistance to a tune of respective 7-fold, 10-fold and 9-fold increase to cypermethrin, deltamethrin and alphamethrin. The synergistic effect of piperonyl butoxide with permethrin was not found to increase mortality to an appreciable extent in the F_5 generation. There was no mortality obtained with 4% DDT in the F_5 generation at 1 hr exposure whereas only with an exposure of 6 hr, 85% mortality was observed. The mosquito was found to remain susceptible to 5% malathion throughout the different generations. The study indicates that owing to the faster development of resistance, the pyrethroids should be judiciously used at the recommended application rates in large scale control programmes and to delay the onset of resistance, alternation or rotation of insecticides with different modes of actions should be followed.

The 5 Students in the II M.Sc. would be submitting their Dissertations shortly on the following topics:

1. Physico-chemical factors which influence *Mansonia* breeding in Shertallai region.
2. Epidemiological investigation of malaria at irrigation development project and its impact on dissemination of malaria to nonendemic area.
3. Studies on the Biology of *Phlebotomus argentipes,* Annondale & Brunetti, 1908 (Diptera: Phlebotomidae). The vector of Indian Kala-azar.
4. Hut scale trial with pyraclofos against mosquito vectors.
5. Determination of the discriminating dose of certain Insecticides for larval stages of Mosquitoes.

The students presented Seminars on the following topics during the year:

1. Host immune responses in Filariasis.
2. Evasion of host immunity in human malarial infection.
3. Diagnostic tools for malaria studies - Past, present and future.
4. Insect viruses and mosquitoes.
5. Malarial drugs and mechanisms of drug resistance.
6. Interaction of macrophages with intracellular parasites.
7. Clinical responses in Human Onchocerciasis - Parasitological and Immunological implications.
8. Effect of pathogens (parasites and viruses) on biology and physiology of vectors.
9. Transovarian and Transtadial transmission of vector borne diseases.

10. Mosquito Cell lines and their applications.
11. Pathophysiology of falciparum malaria in man.
12. Gene cloning in nematodes with special reference to filarial nematodes.
13. The effect of host nutrition on malaria.
14. Non-random host selection by Anopheline mosquitoes.
15. Insect immunity.

Under the Ph.D. programme, the following candidates were awarded the degree during the year:

Sl.No.	Name	Discipline	Month & Year	University
1.	Mr.K.D.Ramaiah	Entomology	Aug, 1990	Pondicherry
2.	Mr.S.L.Hoti	Microbiology	Sep, 1990	Madras
3.	Mr.K.P.Paily	Entomology	Nov, 1990	Pondicherry
4.	Dr.S.P.Pani	Epidemiology	Nov, 1990	Pondicherry

The following candidates have submitted their theses:

1.	Mrs.A.Manonmani	Microbiology	Oct, 1990	Madras
2.	Mrs.Nisha George	Chemistry	Oct, 1990	Pondicherry
3.	Ms.V.Vasuki	Entomology	Oct, 1990	Pondicherry
4.	Mr.M.Kuppusamy	Microbiology	Nov, 1990	Pondicherry
5.	Mr.A.R.Rajavel	Entomology	Nov, 1990	Pondicherry
6.	Mr.R.Srinivasan	Entomology	Nov, 1990	Pondicherry
7.	Ms.M.Jayashree	Entomology	Dec, 1990	Pondicherry
8.	Mr.G.Rajendran	Entomology	Dec, 1990	Pondicherry
9.	Mr.N.PradeepKumar	Entomology	Dec, 1990	Pondicherry

2. MEETINGS/CONFERENCES/SEMINARS/SYMPOSIA ATTENDED

Dr.P.K.Rajagopalan, Former Director, attended the WHO Steering Committee Meeting on Filariasis from 13-16 March 1990 at Geneva. As an adviser, he attended the WHO Informal Consultation meeting on Field Research for Lymphatic Filariasis including Epidemiological Evaluation of Vector Control in Geneva from 21-26 May 1990. Attended the Informal Consultation Meeting of Pesticides in Industries from June 12-25, 1990 at WHO, Geneva. Attended the International Congress of Parasitology held at Paris, France from Aug. 20-25, 1990. Also visited U.K. on the invitation of the Imperial College, Cambridge University and the Liverpool School of

Tropical Medicine, London. He attended the WHO Steering Committee Meeting on Filariasis from 25-28 Sept., 1990 at Geneva.

Dr.P.K.Rajagopalan, also participated in the Seminar on Future Research Needs on Lymphatic Filariasis held at the Vector Control Research Centre, Pondicherry, during 8-10th October, 1990. Dr.P.K.Das, Deputy Director, Dr.K.Balaraman, Dr.K.N.Panicker and Dr.S.P.Pani, Assistant Directors,Dr.S.Sabesan and Dr.P.Jambulingam, Senior Research Officers, Dr.K.Krishnamoorthy and Dr.T.Mariappan, Research Officers, Dr.K.D.Ramaiah, Assistant Research Officer, Mr.S.Subramanian and Mr.P.Vanamail, Technical Officers and Mr.A.Manoharan and Ms.A.Srividya, Statistical Assistants, also participated in the Seminar.

Dr.V.Dhanda, Director, attended the 6th Annual Convention of the Indian Virological Society, sponsored by National Institute of Virology, at Pune from 17-19th December, 1990 and served as a Treasurer in the Organising Committee.

Dr.P.K.Das, Deputy Director, was an invited speaker on Environment and Health in the workshop for training resource persons drawn from voluntary organizations, organised by the Centre for Environmental education at Pondicherry, from 18-22nd August, 1990. He attended a Seminar on Viruses and Vaccines for computers held at, JIPMER, Pondicherry, on 21st October, 1990. He also attended the international colloquim of the Prince Leopold Institute of Tropical Medicine, Belgium from 13-14th December, 1990.

Dr.K.N.Panicker, Assistant Director, was invited to speak on Training modules - Principles, to the Senior Medical Officers of the South-East Asian countries, National Institute of Nutrition, Hyderabad, on 1st February, 1990. He continues to be the patron of the FILCO movement at Shertallai. He also served on the faculty of Community Medicine, Alleppey Medical College, Alleppey.

Dr.S.P.Pani, Assistant Director attended the annual conference of the Indian Medical Association, Pondicherry State Branch held at JIPMER, Pondicherry during 15-16th January, 1990. He attended the Southern Regional Conference of the association of Physicians of India at Kodaikanal from 10-12th August, 1990. He also attended a Seminar on Viruses and Vaccines for Computers held at JIPMER Pondicherry on 21st October, 1990.

Dr.S.L.Hoti, Research Officer, attended a course on Modern approaches in bioprocess technology of enzymes and fuels at the School of Biological Sciences, Madurai Kamaraj University, Madurai from 20th December 1990 to 7th January 1991.

Dr.S.Sabesan, Senior Research Officer and Mrs.Ambilikumar, Research Assistant, continue to be the members of Advisory Board of FILCO movement at Shertallai.

3. MEETING ORGANIZED DURING THE YEAR

An International Seminar on Future Research Needs in Lymphatic Filariasis was organized at this Centre during 8-10 October, 1990. Scientists from abroad and India along with the scientists of this Centre participated. The main aim of this seminar is to review the current knowledge and to highlight the research priorities, with particular reference to the situation prevailing in India. This seminar was very useful to the young scientists to exchange their knowledge and to know the latest techniques prevailing all over the world. The outside participants were:

Dr.D.A.P.Bundy, Director, Field Operations, Parasitic Epidemiology Research Group, Department of Pure and Applied Biology, Imperial College, London, Dr.Bryan T Grenfell, Lecturer in Zoology, Department of Zoology, Cambridge University, Cambridge, Prof.W.W.Macdonald, Liverpool School of Tropical Medicine, Department of Medical Entomology, Liverpool, U.K, Dr.E.A.Ottesen, Chief, Laboratory of Parasitic Diseases, National Institute of Allergy & Infectious Diseases, National Institutes of Health, U.S.A, Dr.C.P.Ramachandran, Secretary, SC/FIL, Division of Tropical Diseases Control, World Health Organization, Switzerland, Dr.M.K.Das, Regional Medical Research Centre, Bhubaneswar, Dr.A.P.Dash, Senior Research Officer, Regional Medical Research Centre, Bhubaneswar, Dr.B.C.Harinath, Head, Department of Biochemistry, Mahatma Gandhi Institute of Medical Science, Wardha, Dr.S.Jamal, Chief, Filariasis Clinical Research Unit, Thanjavur Medical College Hospital, Thanjavur, Dr.S.K.Kar, Regional Medical Research Centre, Bhubaneswar, Dr.J.C.Katiyar,

Central Drug Research Institute, Lucknow, Dr.M.V.V.L.Narasimham, Director, National Malaria Eradication Programme, Delhi, Dr.R.K.Shenoy, Professor and Head, Department of Medicine, T.D. Medical College Hospital, Alleppey, Prof.V.Vijayasekharan, Addl. Professor of Clinical Pharmacology, Institute of Pharmacology, Madras Medical College, Madras, Dr. V. Kumaraswamy, Asst. Director, Tuberculosis Research Centre, Madras, Dr. B.V. Rao, Retd. Prof. of Parasitology, Andhra Pradesh Agricultural University, Tirupati, Dr.M.K.K. Pillai, Prof. of Zoology, University of Delhi, Delhi, Dr.N.L.Kalra, National Consultant, National Malaria Eradication Programme, Delhi, Dr. P.K. Ramachandran, Emeritus Scientist, Defence Research & Development Establishment, Gwalior, Dr. V. Dhanda, Director Grade Scientist, National Institute of Virology, Pune, Dr.Subrmania Reddy, Senior Specialist in medicine, General Hospital, Pondicherry and Dr.Venkateswarlu, Junior Specialist in Skin and VD, General Hospital, Pondicherry.

4. LIBRARY

The library services were expanded by the addition of the following number of books, journals and reports etc. during the period under report :-

Books	123
Journals	1
Reports	70
Reprints	1500
Bound Journals	210
Thesis and dissertations	19

The holdings of the library as on 31.12.89

Books	3076
Journals	92
Annual Reports	180
Video Casettes	6
Reprints	4200
Bound Journals	1627
Reports	2000

Dr.P.K.Rajagopalan, former Director, donated from his personal collection 1400 reprints and 100 Books and reports to the library which will be very useful to the scientists working on mosquitoes. The Library continued to provide Current awareness Services, Compilation of bibliography, Inter Library Loan, Reprint request receiving & sending services and Reprographic services to the staff members of VCRC. The library facilities are as usual extended to the researchers who are working on Communicable Diseases. BIOME (Bibliographic Information on Medical Entomology) developed by using keywords were immensely used by the scientists for upto date information.

5. CONSULTANCY SERVICES OFFERED

A field station of the Centre had been functioning at the Neyveli Lignite Corporation, since October 1988 with the objective to prepare a master plan to control mosquitoes, in the Industrial complex by Integrated Vector Management (IVM) techniques. It was realised that the mosquitogenic problems in the operational area are mainly due to man's interference with the environment and a suggestion has been made to establish a seperate vector control unit headed by a Senior Level Entomologist as Mosquito Control Officer. It was also emphasized that Intersectoral collaboration and Community participation, might help in reducing the magnitude of the problem. A detailed Master plan has been prepared and submitted to the Lignite Corporation in October 1990.

6. INFORMATION RETRIEVAL SYSTEM

The Computer facilities at the VCRC are now being extended to the Post Graduate students of Medical Entomology. Training in the application of software packages like Lotus, Multimate, etc., is given in order to help them to do their data analysis and dissertation work. Three new terminals were installed. Apart from the existing software, GLIM (Generalised Linear Interactive Modelling) one of the packages, known for its flexibility is used for enhancing modelling in various fields of research. The most popular and important software packages namely the SPSS and MINITAB were installed, so that all scientific staff could do their statistical analysis in an efficient manner.

7. VISITORS DURING THE YEAR

1. Dr.D.A.P.Bundy, Director, Parasitic Epidemiology Research Group, Department of pure and applied biology, Imperial College, London.

2. Mr.Deep Chandra Joshi, Asst. Development Engineer, NRDC, New Delhi.

3. Dr.V.Dhanda, Director Grade Scientist, National Institute of Virology, Pune.

4. Dr.M.K.Das, Head, Division of Immunology, Regional Medical Research Centre, Bhubaneswar.

5. Dr.A.P.Dash, Senior Research Officer, Regional Medical Research Centre, Bhubaneswar.

6. Dr.S.Easwaramoorthy, Senior Scientist, Sugar Cane Breeding Institute, Coimbatore.

7. Dr.Eric.A. Otteson, Chief, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious diseases, U.S.A.

8. Dr. C.E. Gordon Smith, Wellcome Trust, London.

9. Dr.M.K.Goverdhan, Deputy Director, National Institute of Virology, Pune.

10. Dr.Bryan T.Grenfell, Lecturer, Department of Zoology, Cambridge University, U.K.

11. Dr.B.C.Harinath, Head, Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Wardha, Maharashtra.

12. Dr.S.Jamal, Chief, Filariasis Clinical Research Unit, Thanjavur Medical College, Thanjavur.

13. Dr.N.L.Kalra, National Consultant, National Malaria Eradication Programme, Delhi.

14. Dr.V.Kumaraswamy, Assistant Director, Tuberculosis Research Centre, Madras.

15. Dr.J.C.Katiyar, Head, Division of Parasitology, Central Drug Research Institute, Lucknow.

16. Dr.S.K.Kar, Head, Division of Clinical Medicine, Regional Medical Research Centre, Bhubaneswar.

17. Dr.A.M.Kurup, Chief Research Scientist, Centre for Social Science Research on Leprosy, Wardha, Maharashtra.

18. Mr.K.C.Kankan, Joint Secretory, Ministry of home affairs, Govt.of India.

19. Dr.Lalait Yadav, Zonal Health Officer, Municipal Corporation, Delhi.

20. Prof.W.W.Macdonald, Head, Department of Medical Entomology, Liverpool School of Tropical Medicine, U.K.

21. Dr.M.V.V.L.Narasimham, Director, National Malaria Eradication Programme, Delhi.

22. Dr.J.S.Pillai, Department of Microbiology, University of Otago, New Zealand

23. Dr.R.Prabhakar, Director, Tuberculosis Research Centre, Madras.

24. Dr.M.K.K.Pillai, Prof. of Zoology, Delhi University, Delhi.

25. Dr.P.K.Ramachandran, Emeritus Scientist and Former Director, Defence Research & Development Establishment, Gwalior.

26. Dr.N.Ramakrishnan, National Fellow, Indian Agricultural Research Institute, New Delhi.

27. Dr.N.Rishikesh, World Health Organization, Geneva.

28. Dr.C.P.Ramachandran, Secretary, SC/Fil, Division of Tropical Disease Control, WHO, Geneva.

29. Mr.Rajeshwar Dayal, Dy.Chief Engineer, NRDC, New Delhi.

30. Dr. R. Rajagopal, Deputy Director (Retd)., National Institute of Communicable Diseases, Delhi.

31. Dr.B.V.Rao, Retd.Prof. of Parasitology, Andhrapradesh Agricultural University, Tirupati.

32. Dr.G.P.Singh, National Malaria Eradication Programme, Delhi.

33. Dr.V.Sambasivam, Retd. Director of Health services, Government of Pondicherry.

34. Mr.Srinivasan, Health Secratory, Government of India, Delhi.

35. Dr.R.K.Shenoy, Prof.& Head, Department of Medicine, T.D.Medical College Hospital, Allepey, Kerala.

36. Mr.P.B.Saxena, Senior Deputy Director General (Admn) & Financial Adviser, Indian Council of Medical Research, New Delhi.

37. Dr.Sarala K. Subharao, Asst.Director, Malaria Research Centre, New Delhi.

38. Dr.K.N.Tiwari, Zonal Health Officer, Municipal corporation, Delhi.

39. Dr.V.Vijayasekharan, Addl.Prof. of Clinical pharmacology, Madras Medical College, Madras

40. Dr.Yadav, Municipal corporation, Delhi.

41. Dr.Subramania Reddy, Senior Specialist in Medicine, Government General Hospital, Pondicherry.

42. Dr.Venkateswarlu, Junior Specialist in Skin and VD, Government General Hospital, Pondicherry.

8. TRAINEES DURING THE YEAR

1. Mr.Aniruddha Pramanik, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal - Trained in Insect Pathology.

2. Mr.Anjan Kumar Phukan, Oil India Limited, Duliajan - Trained in Urban Vector control.

3. Dr.Tristram Wyatt, University of Oxford - Trained in Biological control (Prey-predator interaction).

9. PAPERS PRESENTED AT MEETINGS/CONFERENCES/SYMPOSIA

1. P.K.Rajagopalan & D.A.P.Bundy.
   Research perspectives.
   Workshop on "Future Research Needs on Lymphatic Filariasis in India", Vector Control Research Centre, Pondicherry, 8-10 October, 1990.

2. P.K.Rajagopalan & P.K.Das.
   Control perspectives.
   Workshop on "Future Research Needs on Lymphatic Filariasis in India", Vector Control Research Centre, Pondicherry, 8-10 October, 1990.

3. V.Dhanda.
   Recent trends in the spread of Dengue viruses and their vector *Aedes aegypti* in India.
   "6th Annual convention of Indian Virological Society", Campus of the University of Poona, Pune, 17-19 December, 1990.

4. M.A. Ilkal, V. Dhanda, M.M. Hassan, M. Mavale, P.V.M. Mahadev, P.S. Shetty, S.N. Guttikar & K. Banerjee.
   Entomological investigations during outbreaks of Dengue fever in certain villages in Maharashtra state, India.
   "6th Annual convention of Indian Virological Society", Campus of the University of Poona, Pune, 17-19 December, 1990.

5. A.B. Sadeep, U. Pant & V. Dhanda.
   Growth of some arboviruses in the new cell line from *Aedes krombeini* Huang, 1975.
   "6th Annual convention of Indian Virological Society", Campus of the University of Poona, Pune, 17-19 December, 1990.

6. P.K.Das.
   Perspectives on patterns of infection.
   Workshop on "Future Research Needs on Lymphatic Filariasis in India", Vector Control Research Centre, Pondicherry, 8-10 October, 1990.

7. P.K.Das.
   "Community Participation in vector borne disease control. Facts and Fancies".
   International coloquim of Prince Leopold Institute of Tropical Medicine, Belgium, 14-15 December, 1990.

8. K.N.Panicker.
   "Training modules - Principles".
   Guest lecture to the Senior Medical Officers of the South-East Asian countries, National Institute of Nutrition, Hyderabad, on 1st February, 1990.

9. K.N.Panicker & S.Sabesan.
   Socio-economic perspectives.
   Workshop on "Future Research Needs on Lymphatic Filariasis in India", Vector Control Research Centre, Pondicherry, 8-10 October, 1990.

10. K.N.Panicker.
    "Mosquito nuisance"
    Special lecture to the Councellors of Cochin corporation, Cochin, July, 1990.

11. S.P.Pani.
"Natural history of clinical manifestations on Lymphatic Filariasis". Southern Regional Conference of the Association of Physicians, Kodaikanal, 10-12 August, 1990.

12. S.Jamal & S.P.Pani.
"Clinical perspectives".
Workshop on "Future Research needs on Lymphatic Filariasis in India", Vector Control Research Centre, Pondicherry, 8-10 October, 1990.

10. LIST OF PUBLICATIONS

1. P.K.Rajagopalan
Filariasis in India
The Nat. Med. J. of India. 3(1) 1990, 1-4.

2. S.L.Hoti & K.Balaraman
Utility of cheap carbon and nitrogen sources for the production of a mosquito-pathogenic fungus, *Lagenidium*.
IJMR 91, 1990, 67-69.

3. P.K.Rajagopalan, P.K.Das, S.P.Pani, P.Jambulingam, S.S.S.Mohapatra, K.Gunasekaran & L.K.Das.
Parasitological aspects of malaria persistence in Koraput District, Orissa, India.
IJMR 91, 1990, 44-51.

4. P.Goverdhini, S.S.S.Mohapatra, P.Jambulingam & P.K.Das.
Detection of early dividing forms of *Plasmodium falciparum* in peripheral blood.
IJMR 91, 1990, 70-72.

5. M.Kuppusamy & K.Balaraman
Extra cellular hydrolytic enzyme secretion in *B.thuringiensis* H14 and *B.sphaericus* and their significance in media design.
IJMR 91, 1990, 149-150.

6. S.P.Pani, K.Krishnamoorthy, A. .Rao & J.Prathiba.
Clinical manifestations in malayan filariasis with special reference to lymphoedema grading.
IJMR 91, 1990, 200-207.

7. A.M.Manonmani, S.L.Hoti & K.Balaraman.
Characterization and larvicidal activity of indigenous isolates of *Bacillus sphaericus* from natural breeding habitats.
IJMR 91, 1990, 223-227.

8. K.Viswam, R.Srinivasan & K.N.Panicker.
Endurence to starvation by the immatures of *Toxorhynchties splendens*.
IJMR 91, 1990, 228-229.

9. K.P.Paily & K.Balaraman.
Effect of temperature and host-parasite ratio on sex differentiation of *Romanomermis iyengari* (Welch), a mermithid parasite of mosquitoes.
Indian J. Exp. Biol., 28, 1990, 470-474.

10. P.Vanamail, S.subramanian & P.K.Rajagopalan.
A mathematical analysis of various factors involved in transmission of Bancroftian filariasis in Pondicherry.
IJMR 91, 1990, 289-292.

11. S.S.Sahu, K.Gunasekaran, P.Jambulingam & P.K.Das.
Susceptibility status of *Anopheles fluviatilis, A. annularis* and *A. culicifacies* to Insecticides in Koraput District, Orissa.
Indian J. Mal., 27, 1990, 51-53.

12. P.K.Das, K.Gunasekaran, S.S.Sahu, C.Sadanandane & P.Jambulingam.
Seasonal prevalence and resting behaviour of Malaria Vectors in Koraput District, Orissa.
Indian J. Mal., 27, 1990, 173-181.

13. K.Gunasekaran, S.S.Sahu, C.Sadanandane, S.K.Parida, K.P.Patra & P. Jambulingam.
Morphological variations in some Indian Anophelines from Koraput District, Orissa, India.
Indian J. Mal., 27, 1990, 127-138.

14. K.N.Panicker, S.P.Pani, S.Sabesan & K.Krishnamoorthy.
Choice and integration of different approaches to case detection with special reference to Brugian filariasis in South India.
IJMR 91, 1990, 282-288.

15. P.Vana.nail, S.Subramanian, P.K.Das, S.P.Pani & P.K.Rajagopalan.
Estimation of fecundic life span of *W.bancrofti* from a longitudinal study of infection in human population in an endemic area of Pondicherry, South India.
IJMR 91, 1990, 293-297.

16. K.P.Paily.
An improved method of mass culturing *Romanomermis iyengari*, a mermithid nematode parasite of mosquito larvae.
IJMR 91, 1990, 298-302.

17. P.K.Rajagopalan & P.K.Das.
Problems of malaria control in tribal areas.
ICMR Bull 20(5) 1990, 41-46.

18. M.Jayasree, S.Sabesan & K.N.Panicker.
Weedivorous fishes for the control of vectors of Malayan filariasis.
IJMR 91, 1990, 379-381.

19. M.P.Prasad & M.Kalyanasundaram.
Development and evaluation of controlled release formulations of DEPA, the insect repellent.
IJMR 91, 1990, 453-457.

20. M.P.Prasad & M.Kalyanasundaram.
Development of environmentally compatible controlled release formulations of a mosquito larvicide.
IJMR 93, 1991, 51-54.

21. V.Vasuki.
Effect of insect growth regulators on hatching of eggs of three vector mosquito species.
Proc. Indian Acad. Sci. (Anim. Sci.) 99, 6, 1990.

22. M.P.Prasad, Nisha George, V.Vasuki & M.Kalyanasundaram.
Synthesis and insect growth regulating activity of structurally modified benzoylphenylureas.
Indian J. Chem., 29B, 1990, 951-953.

23. P.K.Das, A.Manoharan, A.Srividya, B.T.Grenfell, D.A.P.Bundy & P.Vanamail.
Frequency distribution of *Wuchereria bancrofti* microfilariae in human populations and its relationships with age and sex.
Parasitology (Accepted).

24. K.P.Paily & K.Balaraman.
Infectivity of a mermithid nematode *Romanomermis iyengari* (Welch) in different conductivity levels under laboratory and field conditions.
Indian J. Exp. Biol., (Accepted).

25. M. Kuppusamy & K.Balaraman.
Effect of corn-steep liquor on growth and mosquito larvicidal activity of *Bacillus thuringiensis* var *israelensis* de Barjac 1978 and *B. sphaericus* Neide 1904.
Indian J. Exp. Biol., (Accepted).

26. A. Srividya, S.P.Pani, P.K.Rajagopalan, D.A.P.Bundy & B.T.Grenfell.
The dynamics of infection and disease in bancroftian filariasis.
Trans. Roy. Soc. Trop. Med. & Hyg., (Accepted).

27. B.T. Grenfell, P.K.Das, P.K.Rajagopalan & D.A.P.Bundy.
Frequency distribution of lymphatic filariasis microfilariae in human populations: population processes and statistical estimation.
Parasitology (Accepted).

28. M.Kalyanasundaram, K.N. Panicker, Ambili Kumar, S. Sabesan & C. Sekar.
Toxicological evaluation of deltamethrin during indoor residual treatment.
WHO/VBC (Accepted).

29. A.Srividya, K.Krishnamoorthy, S.Sabesan, K.N. Panicker, B.T. Grenfell & D.A.P.Bundy.
Frequency distribution of *Brugia malayi* microfiolariae in human population.
Parasitology (Accepted).

30. A.Srividya, S.P.Pani, P.K.Rajagopalan, D.A.P.Bundy, B.T.Grenfell.
The dynamics of infection and diseasein bancroftian filariasis.
Trans. Roy. Soc. Trop. Med. & Hyg., (Accepted).

31. S.G.Suguna.
Chromosomal inversions in natural populations of *Anopheles stephensi* in South India.
Cytobios (Accepted).

32. S.Sabesan, N. Pradeep Kumar & K.N.Panicker.
Note on the utility of *Neochetina* spp. (Coleoptera:Curculionidae) in the control of water hyacinth, a host plant for *Mansonioides* breeding.
Entomon (Accepted).

33. R.Velayudhan, Dominic Amalraj, N.Arunachalam & P.K.Das.
Insecticidal activity of carbosulfan (OMS 3022) and Pyraclofos (OMS 3040) against mosquitoes.
J. Com. Dis., (Accepted).

34. S.L.Hoti & K.Balaraman.
Changes in the populations of *Bacillus thuringiensis* H14 and *Bacillus sphaericus* applied to vector breeding sites.
The Environmentalist (Accepted).

35 Vijayan & K.Balaraman.
Metabolites of fungi and actinomycetes active against mosquito larvae.
IJMR (Accepted).

36. A.M.Manonmani, G.Rajendran & K.Balaraman.
Isolation of mosquito-pathogenic *Bacillus sphaericus* and *Bacillus thuringiensis* from the root surface of hydrophytes.
IJMR (Accepted).

37. S.P.Pani, A.Srividya & P.K.Rajagopalan.
Clearance of microfilaraemia following Diethylcarbamazine (DEC) therapy in periodic *W.bancrofti* infection : relation with age, sex, microfilaria count and clinical status.
Trop. Biomed., (Accepted).

38. S.P.Pani, N.Balakrishnan, A.Srividya, D.A.P.Budny & B.T.Grenfell.
Clinical epidemiology of Bacroftian filariasis: Effect of age and gender.
Trans. Roy. Soc. Trop. Med. & Hyg., (Accepted).

11. MISCELLANEOUS PUBLICATIONS OF VCRC

1. K.Balaraman & J.S.Pillai.
A review of biological control research at Vector Control Research Centre, Pondicherry.
MISC. PUBN. V.C.R.C(12), 1990.

2. Project on the control of Malayan Filariasis in Shertallai - Kerala state.
MISC. PUBN. V.C.R.C(13), 1990.

3. P.K.Rajagopalan & S.P.Pani.
Studies on Lymphatic Filariasis at Vector Control Research Centre, Pondicherry. Abstracts of papers published (1975-1990).
MISC. PUBN. V.C.R.C(14), 1990.

4. S.Subramanian, P.Vanamail, A.Manoharan & A.Srividya.
Application of mathematical and statistical techniques to studies on epidemiology and control of Lymphtic Filariasis.
MISC. PUBN. V.C.R.C(15), 1990.

5. Proceedings of a Seminar on Future Research Needs in Lymphatic Filariasis, 8-10 October 1990.
MISC. PUBN. V.C.R.C(16), 1990.

6. P.K.Rajagopalan, K.Krishnamoorthy, K.N.Panicker & S.Sabesan.
Epidemiological assessmemt of different intervention measures for the control of Malayan Filariasis in Shertallai, South India.
MISC. PUBN. V.C.R.C(17), 1990.

7. T.Mariappan, M.Kalyanasundaram & P.K.Das.
Master plan on mosquito control submitted to Neyveli Lignite Corporation, Tamil Nadu.
V.C.R.C., October 1990.

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DIRECTOR